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THE COMPOSITION OF THE TERMINAL SHOOTS AND FRUITS OF TWO VARIETIES OF APPLE IN RELATION TO ROOTSTOCK EFFECTS

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Of recent years much attention has been paid by pomologists and physiologists, both in this country and abroad, to problems relating to the effects of rootstocks on scion varieties of tree fruits. In pomological investigations in this country much progress has been made, and Hatton and his colleagues at East Malling have shown in numerous field trials that, in the case of the apple, certain rootstocks may be relied upon to produce characteristic effects on scion varieties as regards vegetative characters and fruitfulness. It is unnecessary in this paper to detail even the main results since they have been abundantly and clearly described by Hatton (9).

One aspect of the field results, however, is relevant in the present instance. It has been shown that certain so-called rootstock effects, such as leaf scorch susceptibility, appear to be related to nutrient conditions and such observations suggest that other effects, as yet unrelated to specific rootstock qualities or environmental factors, may be functions of nutrition, and hence their causes may be expected to be elucidated by chemical studies.

In the field of plant physiology, Knight (11) found that, although unworked rootstocks showed differences in transpiration rates, these differences were not observable in scion varieties worked upon the rootstocks, and hence it appeared that the influence of the rootstocks on the scion varieties could not be explained on a basis of transpiration differences.

The problem of the possible relationships between rootstock effect and chemical factors has not as yet received much attention. One of us (T.W.) has previously examined trees in cases of leaf scorch where scorching was apparently most severe in association with certain rootstocks and in such cases it appeared that the most severe scorch was accompanied by lower amounts of potash in the organs of the trees, suggesting that the "scorching" stocks were probably relatively poor "potash" feeders. Such observations, however, have been limited to cases of marked potash deficiency and no comprehensive experiments have been carried out hitherto with a view to determining possible relationships between rootstock effects and chemical composition of scion varieties.

In view of the lack of knowledge of this subject, an extensive investigation has been carried out using material from one of the rootstock plots at Long Ashton where detailed pomological data over several years are available. The chemical determinations were carried out on terminal shoots during 1931 and on fruits from crops over the period 1927-31.

The results show that although in this experiment rootstocks produced significant differences as regards certain chemical constituents of terminal shoots and fruits, many of the outstanding rootstock effects could not be explained on the basis of the chemical characters examined.

HISTORICAL.

There are very few investigations relating to the chemical aspects of the rootstock problem which are relevant to the present investigation. Earlier work on the subject has been mainly centred around the question of the ability of various substances to pass the graft union. Of academic interest are the papers by Strasburger (18), Meyer and Schmidt (14), Winkler (21) and Javillier (10) on the transfer of alkaloids across the graft union. Carbohydrate metabolism of grafted plants formed the subject of study by Daniel (4, 5). These investigations concern mainly the transfer across the graft union of substances wholly elaborated within the plants and do not relate to those initially passed from the rootstock to the scion.

Rivière and Bailhache (16) noted on vines that the rootstock affected not only the colour and weight of the fruit but that acidity of the juice, and sugar and ash contents of the juice were also affected. Wallace (20) and Brown (1) have demonstrated rootstock effects upon the composition of the fruit of apple trees.

Daniel and Thomas (6) suggested that grafting affected the intake of minerals. Haas and Halma (8) have carried out an extensive series of analyses of the bark of various citrus trees on different stocks. They concluded that the scion species or variety affected the amount of soluble magnesium in the bark of the stock but they failed to find any appreciable effect of the stock upon the scion. Roach (17) has investigated the effect of stock upon the intake and distribution of the "minor" elements (e.g. lead, molybdenum, etc.) for apples and states that these characters are affected by the stock. Colby (3) has recently analysed apple trees of various American varieties growing upon certain of the Malling rootstocks. Compared with Mall. XII, Mall. IX reduced the phosphorus content of the top shoots by 40 per cent. He suggests that this low phosphorus concentration in the top shoots of trees grafted on to Mall. IX is one of the possible causes of the dwarfing effect which it exerts upon scions worked on to it.

EXPERIMENTAL.

Materials Used in the Investigation.

The rootstock trial plot at Long Ashton provided the material for the investigation. The plot consists of eight trees, each of the three varieties Lane's Prince Albert, Worcester Pearmain and Bramley's Seedling, worked on to each of twelve vegetatively propagated rootstocks, but only the Worcester Pearmain and Lane's Prince Albert varieties are considered here. The trees were planted as maidens during the winter 1919-20. The detailed growth and cropping results on these trees up to 1928 will be published shortly from Long Ashton.

The soil of the plot is mainly derived from Keuper Marl with a thin surface wash of fine sandy material also of Triassic origin, and possesses satisfactory natural drainage. Until 1928, the trees were grown under arable conditions. In that year the plot was grassed down to check vegetative growth and since then the trees have been under a system of grass culture.

The manurial treatment of the plot since planting has been as follows, the rates of application being shown in cwts. per acre.

1920-7. (Each year.)	Nitrate of Soda or Sulphate of Ammonia, 2; Superphosphate, 2; Steamed Bone Flour, 1; Sulphate of Potash, 2.
1928-9.	Sulphate of Potash, 3.
1929-30.	Sulphate of Potash, 3·5; Nitrate of Soda, 3·8.
1930-1.	Sulphate of Potash, 3; Nitrate of Soda, 5; Superphosphate, 3.

It will be seen that the trees have been manured so as to maintain potash supply at a high level, whilst the cultural practices and nitrogenous dressings have ensured adequate but not excessive nitrogen supplies.

The growth and appearance of the trees since planting suggest that there has been no deficiency of nitrogen, phosphate or potash at any time, although prior to grassing down the trees were possibly somewhat over-nitrogenous and vegetative.

During 1931 when the investigation on the shoots was carried out, the Lane's Prince Albert trees were not carrying crops whilst those of the Worcester

Pearmain variety bore heavy crops on all stocks with the exception of Nos. XII and OF5 where the crops were only medium.

Chemical Investigations.

a. Shoots.

Field Sampling. For the purpose of chemical analysis of the shoots, from 30 to 40 leader shoots were gathered for each sample in mid-July. This was done for both varieties growing upon each of the nine rootstocks examined. The shoots were gathered between 9 a.m. and 10 a.m. each day and sampling extended over five days.

The shoots gathered were all typical leader shoots developed in exposed positions and as far as possible not under direct influence of adjacent fruit bearing spurs. Mulay's work (15) shows that the number of shoots gathered is likely to be sufficient to give a representative sample. This was confirmed by a statistical analysis of data obtained for total nitrogen in the leaves of each tree of Lane's Prince Albert on Mall. B stock.

Immediately on cutting, the shoots were wrapped in waterproof paper, conveyed to the laboratory and prepared for analysis.

Laboratory Sampling. In each sample the lengths of individual shoots were measured. The shoots were divided into three portions, leaves, bark + petioles, and wood.

Determinations of nitrogen were made on fresh material. Large samples were used for dry matter determination and the dried materials used for determinations of total ash and ash constituents.

b. Fruits.

Field Sampling. The fruits used have been obtained from samples gathered in connection with storage investigations. The method followed in gathering the samples was to select trees carrying representative crops for each stock and to pick a fairly large proportion of the crop from each tree so that the resultant sample would reflect the general character of the fruits in each case. Only sound fruits were gathered and abnormally large or small fruits neglected.

The whole of the picking was always done during one day under dry conditions, the samples immediately conveyed to the fruit store and the analytical samples drawn without delay.

Laboratory Sampling. Twenty-five fruits, representative of the crop, were taken for analysis and determinations made on the fruit pulp. Total nitrogen was determined on the fresh material, sugars and acids on material preserved in alcohol, and ash and ash constituents on oven dried material.

METHODS OF ANALYSIS.

1. *Total Nitrogen* was estimated by the Kjeldahl method.

2. *Dry Matter.*

(a) *Shoots.* The major portion of the water was evaporated in a water oven and the material finally dried at 100° C. to constant weight.

(b) *Fruits.* A requisite amount of material was dried for seven days at 70° C. This time is sufficient for approximate constancy of weight to be attained and the temperature not sufficiently high for appreciable decomposition.

3. *Total Ash.* The dried material was ashed at as low a temperature as possible, frequent leachings with hot water being carried out. The final ash was almost pure white in colour.

4. *Ash constituents in Ash Solution.*

(a) *Calcium* was precipitated as oxalate after removal of silica, iron and aluminium from the ash. The precipitate of calcium oxalate, after solution in dilute sulphuric acid, was titrated with standard potassium permanganate.

(b) *Magnesium* was precipitated as magnesium ammonium phosphate from the calcium free solution. The precipitate was ignited to magnesium pyrophosphate and weighed as such.

(c) *Potash* was estimated on a separate aliquot as perchlorate after removal of sulphate etc. by the usual methods.

(d) *Phosphate* was precipitated as ammonium phosphomolybdate and the precipitate titrated with standard alkali and acid.

5. *Sugars.* The alcohol preserved material was continuously extracted with 85 per cent. alcohol for twenty-four hours in a Soxhlet extraction apparatus, an excess of calcium carbonate being kept in the receiving flask to prevent inversion of sucrose. After removal of the alcohol in vacuo, the residue was taken up in water and the reducing sugars estimated, after clarification, by titration with Fehling's solution, using methylene blue as indicator (12).

Reducing sugars are expressed as invert sugar.

Sucrose was estimated by titration after inversion for ten minutes with 10 per cent. boiling citric acid solution.

6. *Titratable acidity.* The alcohol preserved material was continuously extracted as for sugars but without the addition of calcium carbonate. After removal of the alcohol in vacuo, the residue was taken up in water and an aliquot titrated with standard alkali, using phenolphthalein as indicator. Acidity was calculated as malic acid and is expressed as such.

POMOLOGICAL INVESTIGATIONS.

The pomological records utilized to evaluate rootstock effect in the present instance have been mainly collected by the pomological staff of the Long Ashton Station.

In collecting data, records for individual trees have been kept separate so that it has been possible to determine the amount of variation within the group of trees comprising each rootstock series.

The more important measurements available for the study from the general pomological records were: total annual extension growth since planting to 1926 for trees of Lane's Prince Albert and to 1928 for those of Worcester Pearmain; heights and spreads to 1926; girth of the main stems; number of blossom trusses; number of fruits "set", determined after June drop; number and weight of fruits harvested.

Of these, the data relating to total annual shoot growth and total crop weights to 1926 were selected for examination as expressing vigour and cropping performance of the trees in the earlier years of their history.

The following additional records were collected in 1931 from all trees of both varieties for the special purpose of the investigation: extension growth of selected terminal shoots to mid-July and for the whole season.

RESULTS.

The data obtained for both the chemical and pomological sides of the investigation are too extensive to present in any detail and hence only summarized tables of the analyses of the results are given.*

Where the data have been analysed statistically, the standard of significance used has been that the chances against a result being fortuitous must be at least twenty to one.

1. CHEMICAL RESULTS FOR TERMINAL SHOOTS.

Estimations of total nitrogen, dry matter, total ash, calcium, magnesium, potassium, sodium,† iron, aluminium, manganese, phosphate and silica were made on the terminal shoots of the two varieties growing upon each of the nine rootstocks, Mall. I, Mall. II, Mall. IV, Mall. V, Mall. VII, Mall, IX, Mall. X, Mall. XII (B)‡ and O.F.5. The procedure followed in presenting the summary of the chemical results has been to arrange the stocks in descending order of magnitude in respect of the content of the elements considered in the shoots of the scion on the individual stocks (Table I). The two varieties and the three classes of material analysed (leaves, bark and wood) are treated separately. For the extreme cases the actual values obtained are given in brackets. We thus have the order of the stocks in respect of any one constituent together with the range of values. When two or more stocks are joined above by a tie, it indicates that they have identical values. The figures have all been expressed on a percentage fresh weight basis. This seemed to be preferable to a dry weight basis, especially as sampling extended over several days, Chibnall (2) and Mason and Maskell (13).

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* The detailed data have been filed at Long Ashton and are available for inspection.

† Results for Na, Fe, Al, Mn, and SiO_2 not considered in detail in this paper.

‡ Mall. XII rootstock Worcester Pearmain only and Mall. B. rootstock Lane's Prince Albert only.

In addition, ratios between the amounts of certain of the chief ash constituents and nitrogen were examined, but only total ash to nitrogen, and potash to nitrogen are considered in the table.

TABLE I.

Data for Shoots.

Showing rootstocks arranged in descending orders of magnitude for various constituents and ratios of constituents.

a. *Variety: Worcester Pearmain.*

Dry Matter	Leaves	OF5 (35·4) V $\overline{\text{II IX}}$ $\overline{\text{I VII}}$ IV XII X	(32·2)
	Bark	V (34·6) $\overline{\text{IV I}}$ II OF5 IX VII X XII	(32·8)
	Wood	II (43·8) $\overline{\text{X IV}}$ I V IX OF5 VII XII	(41·2)
Total Nitrogen	Leaves	V (0·965) I X II IV IX VII XII OF5	(0·837)
	Bark	$\overline{\text{OF5 (0·464) V}}$ II XII VII I IV X IX	(0·418)
	Wood	V (0·509) X XII VII II I OF5 IX IV	(0·410)
Total Ash	Leaves	XII (2·39) OF5 I IX V II X VII IV	(1·79)
	Bark	IX (2·46) I XII OF5 IV VII II X V	(2·12)
	Wood	IX (0·96) XII V $\overline{\text{VII I}}$ OF5 IV II X	(0·80)
Lime (CaO)	Leaves	XII (0·684) V I IX II OF5 VII X IV	(0·406)
	Bark	I (1·006) IX XII VII II OF5 IV X V	(0·771)
	Wood	IX (0·316) I VII XII X II V IV OF5	(0·202)
Magnesia (MgO)	Leaves	II (0·158) XII V I IX $\overline{\text{IV OF5}}$ X VII	(0·104)
	Bark	II (0·131) XII V IX OF5 IV I X VII	(0·090)
	Wood	XII (0·039) V $\overline{\text{X VII OF5 II}}$ $\overline{\text{IV IX}}$ I	(0·028)

Potash (K_2O)	Leaves	OF5 (0·645) IX IV XII I X II V VII (0·467)
	Bark	IX (0·532) OF5 XII IV VII I II X V (0·328)
	Wood	OF5 (0·234) IX XII V VII I IV X II (0·201)
Phosphate (P_2O_5)	Leaves	XII (0·215) I V X OF5 VII II IX IV (0·172)
	Bark	VII (0·113) II V I XII OF5 IV IX X (0·095)
	Wood	IX (0·100) IV V VII II X I XII OF5 (0·081)
Total Ash : Nitrogen	Leaves	XII OF5 IX I II VII X V IV
	Bark	IX I IV VII XII X OF5 II V
	Wood	IX IV XII OF5 I VII II V X
Potash : Nitrogen	Leaves	OF5 IX XII IV X I II VII V
	Bark	IX IV VII XII OF5 I II X V
	Wood	IX OF5 IV XII VII I V II X

b. *Variety: Lane's Prince Albert.*

Dry Matter	Leaves	I (37·5) V VII II IV IX X OF5 B (31·7)
	Bark	IV (35·5) V II VII IX I X OF5 B (31·5)
	Wood	IX (40·4) VII X OF5 II B I IV V (35·3)
*Total Nitrogen	Leaves	VII (1·108) V II IX X B OF5 IV (0·978)
	Bark	V (0·497) IV VII OF5 II B X IX I (0·416)
	Wood	V (0·664) B VII OF5 II IX X IV I (0·483)
Total Ash	Leaves	B (2·66) VII IX X I OF5 II IV V (1·98)
	Bark	I (2·52) IX X B OF5 II IV VII V (2·09)
	Wood	X (0·76) VII IV IX I B II OF5 V (0·66)
Lime (CaO)	Leaves	B (0·751) IX II VII V X I OF5 IV (0·463)
	Bark	IX (0·817) X II VII B OF5 I V IV (0·724)
	Wood	IX (0·150) X VII I II IV OF5 V B (0·115)

Magnesia (MgO)	Leaves	B (0·164) IX II V I VII OF5 X IV (0·098)
	Bark	II (0·130) B I V IX OF5 IV VII X (0·090)
	Wood	X (0·046) II B I V IX IV VII OF5 (0·035)
Potash (K_2O)	Leaves	B (0·730) OF5 IV I X VII IX II V (0·523)
	Bark	I (0·580) OF5 B IV II IX VII X V (0·424)
	Wood	IX (0·286) X I VII B OF5 IV V II (0·207)
Phosphate	Leaves	B (0·174) X V IX VII IV I OF5 II (0·140)
	Bark	VII (0·119) I OF5 IX IV B II V X (0·101)
	Wood	VII (0·091) IV IX X I V II B OF5 (0·066)
*Total Ash : Nitrogen	Leaves	B IX OF5 X IV VII II V
	Bark	I IX B X VII OF5 IV II V
	Wood	I IV X IX II VII OF5 B V
Potash : Nitrogen	Leaves	OF5 B IV X IX VII II V
	Bark	I B OF5 IV II IX X VII V
	Wood	I IX X IV B VII OF5 II V

These analytical data were subjected to statistical treatment and three main lines of attack followed:

(a) The relation between pairs of mineral constituents as per cent. of the total ash was examined by means of dot diagrams. Where any evidence of a correlation was obtained, correlation coefficients were extracted. There was a possibility that when a correlation was obtained it might be due solely to differences in the composition of the ash of the different tissues. Hence, only when a correlation holds for each of the three classes of material analysed is it considered significant.

The results obtained, together with standard errors, are appended below.

		r.
i. K_2O and CaO	All the Data	$-0{\cdot}8834 \pm 0{\cdot}0298$
	Ash of Wood	$-0{\cdot}8849 \pm 0{\cdot}0525$
	Bark	$-0{\cdot}7790 \pm 0{\cdot}0927$
	Leaves	$-0{\cdot}8103 \pm 0{\cdot}0811$

---

* No value for stock I.

ii. K_2O and MgO	All the Data	+0·2658 ± 0·1262
iii. CaO and P_2O_5	All the Data	−0·9017 ± 0·0257
	Ash of Wood	−0·4017 ± 0·1978
	Bark	−0·3810 ± 0·2008
	Leaves	−0·2125 ± 0·2236
iv. K_2O and P_2O_5	All the Data	+0·7462 ± 0·0601
	Ash of Wood	+0·4839 ± 0·1758
	Bark	+0·1157 ± 0·2325
	Leaves	−0·2514 ± 0·2205
v. CaO and MgO	All the Data	−0·2916 ± 0·1239

Only for potash and lime is there any correlation which can be considered significant.

(b) The data were then considered from the point of view of possible correlations between the amounts of each element in the different classes of material analysed. Values for r were obtained as under:

i. Total Nitrogen	Leaves and Wood	+0·8670 ± 0·0602
	Leaves and Bark	+0·7260 ± 0·1151
	Bark and Wood	+0·7599 ± 0·1025
ii. Total Ash	Leaves and Wood	−0·1212 ± 0·2322
	Leaves and Bark	+0·3919 ± 0·2031
	Bark and Wood	+0·2116 ± 0·2251
iii. K_2O	Leaves and Wood	+0·5961 ± 0·1519
	Leaves and Bark	+0·6900 ± 0·1235
	Bark and Wood	+0·4702 ± 0·1930
iv. CaO	Leaves and Wood	−0·2007 ± 0·2238
	Leaves and Bark	+0·1441 ± 0·2308
	Bark and Wood	+0·6935 ± 0·1223
v. MgO	Leaves and Wood	+0·0829 ± 0·2341
	Leaves and Bark	+0·7860 ± 0·0901
	Bark and Wood	+0·2386 ± 0·2223
vi. Fe_2O_3	Leaves and Wood	+0·1185 ± 0·2324
	Leaves and Bark	+0·1222 ± 0·2354
	Bark and Wood	+0·6704 ± 0·1298

vii.	Mn_3O_4	Leaves and Wood	−0·3255 ± 0·2107
		Leaves and Bark	−0·1562 ± 0·2300
		Bark and Wood	+0·5259 ± 0·1705
viii.	P_2O_5	Leaves and Wood	+0·1604 ± 0·2297
		Leaves and Bark	−0·4409 ± 0·1900
		Bark and Wood	+0·2909 ± 0·2158

The positive correlations between the nitrogen contents of the three sets of material and the two significant correlations for potassium are of most interest. Calcium, iron and manganese in bark and wood are positively correlated also, whilst magnesium in leaves and bark show a significant positive correlation. Phosphate in the leaves and bark also suggests a negative correlation.

(c) The data were further analysed using Fisher's Z test (7). In order to obtain a symmetrical table, the data for Worcester Pearmain on stock Mall. XII and for Lane's Prince Albert on Mall. B were omitted. Differences due to the presence of a crop on the Worcester Pearmain trees will appear as a scion difference. The two varieties are not affected equally or necessarily in the same direction by the different stocks as regards their growth character (see pages 14 to 16). If there is any such differential effect upon chemical characters then the effect of this will be to increase the remainder value and so decrease the two values for Z, viz.:

Z Scion – Remainder and
Z Stock – Remainder.

There is a possibility therefore that significant differences due to stock may be masked in this way. The complete variance tables are not reproduced, but values for Z Stock – Remainder are appended below, together with values for a 1 per cent. point and a 5 per cent. point.

Values for Z Stock – Remainder.

1% Point.	5% Point.	
0·9789	0·6668	for all cases except Total N in Leaves.
1·0680	0·7274	for Total N in Leaves.

Total Nitrogen:	Leaves	1·1279
	Bark	0·5385
	Wood	0·6410
Total Ash:	Leaves	0·0244
	Bark	1·3645
	Wood	0·3897

Potash (K_2O):	Leaves	0·6645
	Bark	0·3093
	Wood	1·7379
Phosphate (P_2O_5):	Leaves	0·5423
	Bark	1·2322
	Wood	0·7510
Calcium oxide (CaO):	Leaves	0·9813
	Bark	0·4913
	Wood	0·3598

If a value for Z equal to the 5 per cent. point be taken as significant, then only for total nitrogen in leaves, total ash in bark, potash in wood, phosphate in bark and wood, and calcium in leaves are there any significant stock effects. In addition, there is for total nitrogen in the wood and bark, potash in leaves, phosphate in leaves and calcium in bark, a stock effect which approaches significance.

2. CHEMICAL DATA ON FRUITS.

The data obtained consist of estimations of dry matter, total nitrogen, acidity, reducing sugar, sucrose, total ash, potash and phosphate for the fruits of both varieties growing upon each of twelve rootstocks, Mall. I, Mall. II, Mall. III, Mall. IV, Mall. V, Mall. VI, Mall. VII, Mall. IX, Mall. X, Mall. XII (B), Mall. XIII and O.F.5. The analyses are of fruits for the seasons 1928-31 inclusive for Worcester Pearmain and 1927-30 inclusive for Lane's Prince Albert. The method of treatment of the data has been to calculate mean values for the content of each constituent and then to extract standard errors. Owing to the small number of observations the standard errors will not have quite their normal significance. The data are summarized below. The two varieties are treated separately and the stocks arranged in descending orders of magnitude. The values for the extreme cases are shown in brackets. The inclusion of any stock in a square bracket indicates that the numbers of observations are too few to allow of any statement as to the significance of the mean value. The stocks are shown in significant groups, i.e. the value at the beginning of one arrow differs significantly from the value at the beginning of the next arrow.

It will be seen that with both varieties there is a significant effect in respect of all the constituents estimated with the exception of reducing sugars and potash in the Worcester Pearmain, and sucrose and total ash in the Lane's Prince Albert. It will be noted, however, that in the majority of cases there are only two significant groups and the influences exerted by rootstocks on the content of the constituents estimated are thus relatively small.

Table II.

Data for Fruits.

Showing rootstocks arranged in descending orders of magnitude in significant groups for the various constituents expressed on a per cent. of fresh weight basis.

a. *Variety: Worcester Pearmain.*

Nitrogen	VII (0·0647) XII III XIII OF5 II I IX IV ↓ V X VI (0·0511)
Acid	XII (0·479) OF5 XIII IV X IX VI ↓ I III VII V ↓ II (0·375)
Reducing Sugars	IX (8·77) VII III II X XIII IV XII I VI V OF5 (7·87) ↓
Sucrose	IX (2·72) XII I II VI OF5 ↓ VII IV V III X XIII (1·82)
Total Sugars	IX (11·49) VII II ↓ I XII III IV VI V X OF5 XII (10·08)
Total Ash	XII (0·27) OF5 XIII III X I [VI] VII IX IV ↓ II V (0·22)
Potash	OF5 (0·15) XII III XIII I [VI] VII IX X II IV V (0·12)
Phosphate	V (0·022) I XII III IV II V VII IX X XIII ↓ OF5 (0·015)
Dry Matter	IX (15·45) VI VII IV III ↓ OF5 XII II V I XIII ↓ X (13·73)

b. *Variety: Lane's Prince Albert.*

Nitrogen	B (0·083) OF5 VII XIII ↓ VI IX V IV I X II ↓ III (0·052)
Acid	V (1·09) B OF5 I IV ↓ VI II VII III XIII IX X (0·91)
Reducing Sugars	IX (6·97) VII II X ↓ VI III V IV I XIII OF5 B (6·00)
Sucrose	IX (2·00) I III XIII X VI II VII V IV B [OF5] (1·38)
Total Sugars	IX (8·97) ↓ III X II VI VII I XIII V ↓ IV B [OF5] (7·50)
Total Ash	OF5 (0·22) B XIII I II IV III V VI IX X (0·18)
Potash	OF5 (0·111) B IV VI I II ↓ III XIII VII ↓ IX V X (0·089)
Phosphate	XIII (0·023) V B OF5 IX ↓ I II VI X IV VII III (0·015)
Dry Matter	IX (11·56) VII ↓ VI XIII X III V IV I II OF5 B (10·30)

3. POMOLOGICAL DATA.

(*a*) *Tree Size and Vigour.*

These data refer only to the nine stocks, the shoots of which were examined chemically.

(i.) *Tree Size as shown by metres of total wood growth.* For Worcester Pearmain we have available figures of total wood growth per tree in metres for two periods from planting, to 1926 and 1928 respectively. The stocks are shown arranged in descending order of vigour, the various significant groups being indicated by the arrows.

Growth to 1926	XII (209·0) OF5 IV I II V X VII IX (57·2)
Growth to 1928	XII (394·5) OF5 I IV II X V VII IX (96·3)

For Lane's Prince Albert similar data to 1926 only are available.

Growth to 1926	I (272·0) OF5 B II IV V X VII IX (50·3)

(ii.) *Shoot Growth in 1931.* For the purpose of estimating shoot growth conditions during 1931, eight (usually) leader shoots upon each of the trees of the two varieties were measured when growth had ceased for the season. From these data the stocks are arranged in descending order of magnitude, the significant groups being indicated.

Worcester Pearmain	V (55·6 cms.) XII OF5 I II X IV VII IX (31·1 cms.)
Lane's Prince Albert	B (83·9 cms.) VII OF5 V I II IV X IX (48·9 cms.)

This order of vigour differs somewhat from the order of vigour of the stocks based upon the amount of shoot growth which had taken place by mid-July at the date of sampling. On this latter basis the order of the stocks is as under.

Worcester Pearmain	V (38·0 cms.) OF5 XII I X IV II VII IX (27·3)
Lane's Prince Albert	OF5 (65·1) B V IV I VII II X IX (47·0)

The agreement for the two dates is fairly good for the Worcester Pearmain, but with the Lane's Prince Albert, Mall. VII, which was low in vigour in mid-July, took second place by the end of the season. This was due to the late growth shown by the trees on this stock. Mall. VII in the case of Lane's Prince Albert appeared to be of abnormal vigour in 1931, since before that time its vigour had been low, whilst subsequent to 1931 it has again exerted a dwarfing effect.

(*b*) *Cropping Data.*

For both varieties the stocks are arranged in order of the weight of fruit produced to 1926 and to 1928. Weights are in pounds per tree and the significant groups are indicated. No account is taken of varying tree size.

Worcester Pearmain.

Weight of fruit to 1926 II (7·4) IX IV I VII V X OF5 XII (0·35)

Weight of fruit to 1928 II (41·8) IV IX I V VII X OF5 XII (15·9)

Lane's Prince Albert.

Weight of fruit to 1926 IV (20·8) II I VII IX V OF5 X B (0·08)

Weight of fruit to 1928 I (132·9) IV II X OF5 B V VII IX (57·8)

(*c*) *Precocity.*

A numerical expression of precocity presented some difficulty. Finally, for the present purpose, precocity was taken to be indicated by the weight of fruit produced per hundred metres of shoot growth up to 1926. When values for precocity are calculated in this way the stocks fall in order as under, the significant groups being indicated.

Worcester Pearmain IX (11·4) II IV I VII V X OF5 XII (0·17)

Lane's Prince Albert IX (18·8) II IV VII V I X OF5 B (0·04)

(*d*) *Periodicity in Shoot Growth.*

A possible relation of period of seasonal elongation of shoots to flower bud formation has been indicated by Swarbrick (19).

In the present investigation three trees of each of the two varieties growing upon each of the nine rootstocks were selected and eight typical leader shoots chosen upon each tree. Measurements of the lengths of these shoots were taken at intervals throughout the growing season. Growth rates and relative growth rates were calculated for each period between the dates of measurement. As mid-July is believed to be a critical period in flower bud initiation under Long Ashton conditions, only the growth data for this period are given. When the stocks are arranged in order of growth rates for mid-July they fall in order as under.

Worcester Pearmain.

I. Growth rates (in growth per day) for period July 15th to July 23rd.

XII (0·68) V X $\overline{\text{I II VII}}$ OF5 IV IX (0·20)

II. Relative growth rates (in growth per cm. per day).

XII (0·0175) X V OF5 VII II I IV IX (0·0075)

Lane's Prince Albert.

I. Growth rates (in growth per day) for period July 6th to July 18th.

B (0·83) VII V OF5 I IV X II IX (0·42)

II. Relative growth rates (in growth per cm. per day).

VII (0·0162) $\overline{\text{V X}}$ B IX OF5 I IV II (0·0105)

DISCUSSION.

1. CHEMICAL DATA.

(*a*) *Shoots.*

The data for the two varieties show considerable and fairly consistent differences in shoot composition but as it is not possible to separate varietal differences from differences due to the presence of a crop on the Worcester Pearmain, this point will not be discussed.

The question of stock influence is not complicated to the same extent by the crop factor though differences in cropping in previous years may introduce a disturbing factor here.

Analyses of the data for the shoots by means of Fisher's Z test indicated that shoot composition was significantly affected in respect of several constituents by the rootstocks. In view of these results, even though the data do not allow of testing the significance of differences between individual stocks for each variety it seemed worth while to classify the stocks in high and low groups for various constituents and ratios of constituents where the results for leaves, bark and wood indicated consistent tendencies. Table III shows the grouping of stocks on this basis.

(*b*) *Fruits.*

The significance of the results obtained for the various constituents in the fruits has been indicated in Table II. In view of the fact that for almost every constituent with each variety significant groups occurred, the orders for the stocks in Table II have been arranged into high and low groups for each constituent in Table IV. In this latter table, the presence of a stock in a high or low group indicates that the stock occurs in the upper or lower divisions of the orders shown for the various constituents for both varieties in Table II.

TABLE III.

Showing the grouping of rootstocks on basis of high and low values for various chemical constituents or ratios of constituents.

Constituent or Ratio of Constituents.	High.	Inclined to be High.	Low.	Inclined to be Low.
(*a*) *Worcester Pearmain*				
Dry Matter	**II**.	V	*XII, VII	
Total Nitrogen	**V**	II	IX	
Total Ash	XII, **IX**	**I**	**X, II**	IV
Lime (CaO)	XII, I, **IX**		**IV, OF5**	II, X
Magnesia (MgO) ..	*XII, **V**		**IV**	**VII,** X, OF5
Potash (K_2O)	OF5, IX, XII		X, **II**	**V,** VII
Phosphate (P_2O_5) ..	V			OF5
$\frac{\text{Total Ash}}{\text{Nitrogen}}$	**IX**	I	**V, II,** X	
$\frac{\text{Potash}}{\text{Nitrogen}}$	IX, **IV,** *XII	OF5	**V, II,** I	X
(*b*) *Lane's Prince Albert*				
Dry Matter	VII	**II**	*B	
Total Nitrogen	VII, **V**	**II**	†I	X
Total Ash		X, **IX, I**	V, **II**	OF5
Lime (CaO)	**IX,** VII	II	**IV, OF5**	V
Magnesia (MgO) ..	*B, II	I, **V**	OF5, **IV, VII**	
Potash (K_2O)	I		**V**	**II**
Phosphate (P_2O_5) ..	IX	VII	II	
$\frac{\text{Total Ash}}{\text{Nitrogen}}$	**IX,** X		**V**	**II**
$\frac{\text{Potash}}{\text{Nitrogen}}$	**IV**	*B	**V,** VII	**II**

Heavy type denotes agreement between the two varieties.

* Denotes agreement for Stocks XII and B between the two varieties.

† No value for Leaves.

In order to bring out prominently the stocks occupying the highest and lowest values respectively, those occupying the first three or last three places for both varieties are specially marked in Table IV. In the cases of stocks XII and B, which only occur with one variety, these are given a distinctive mark as a reminder of this fact.

TABLE IV.

Showing the grouping of rootstocks on basis of high and low values for various chemical constituents in both varieties.

Chemical Constituent.	Rootstocks. High Values.	Rootstocks. Low Values.
Dry Matter	**IX, VII, VI**	*B, I, II, OF5
Total Nitrogen	**VII,** *XII, *B, XIII, OF5	**X,** V, IV
Acidity	**OF5,** *XII, *B, IX	III, II, VII
Reducing Sugars	**IX, VII,** II, X	**OF5,** *B, I, XII
Sucrose	**IX, I,** *XII	*B, V, IV
Total Sugars	**IX,** II, VII	**OF5,** *B, IV, V, XIII
Total Ash	**OF5, XIII,** *XII, *B	**V**
Potash (K_2O)	**OF5,** *XII, *B	**V**, IX, X
Phosphoric Acid (P_2O_5)	**V,** *XII, *B	**X**, VII

Heavy type denotes occurs within top or bottom three values for both varieties.

* Denotes occurs within top or bottom three values for one variety considered.

2. POMOLOGICAL DATA.

It is necessary to discuss certain of these data briefly before attempting to relate chemical characters with growth characters.

(*a*) *Tree Size and Vigour.*

A classification of stocks according to vigour of the worked trees up to 1926 separates IX as a dwarfing stock. The stocks VII, X and V constitute a significant group exerting a dwarfing effect for both varieties whilst by 1928, VII, with Worcester Pearmain, forms a significant group, being now definitely separated from X and V. No further subdivision of stocks on the basis of growth up to 1926 is possible but the figures for total wood growth up to 1928 for Worcester Pearmain separate stocks XII and OF5 as being more vigorous than the other stocks.

(*b*) *Shoot Growth in 1931.*

When the stocks are arranged in order of vigour, using shoot growth in 1931 as the basis of classification, they differ in certain respects from the classification based on total wood growth up to 1926.

Measurements of growth of leader shoots in earlier years on a number of these trees gives a classification of stocks which agrees with the order based on total wood growth for that period (19). Growth of leader shoots during 1931 does not therefore show the same stock effects as did growth up to 1926. Stock V is now relatively more vigorous than in earlier years and especially is this so with Worcester Pearmain. Stock IV on the other hand shows a slight relative decrease in vigour for both varieties. Stock VII agrees with its earlier position for Worcester Pearmain but with Lane's Prince Albert now occupies the second place for vigour. Stock I with Lane's Prince Albert shows a considerable relative decrease in vigour.

A classification of stocks based on the growth of leader shoots by mid-July, i.e. at the date of sampling for chemical analysis, shows a few discrepancies when compared with the classification based on growth of leader shoots for the whole season. The only discrepancy of any magnitude, however, is furnished by stock VII for Lane's Prince Albert. The shoots of the trees of this variety on this stock continued to grow very late in the season and so by the end of the season, the stock, on a basis of shoot growth, appeared as a vigorous one.

(*c*) *Cropping Data.*

The cropping data indicate that the rootstocks have significantly affected the weight of fruit borne by the trees. The trees are so affected in size by the several rootstocks that direct comparison of yields by the trees on the different stocks furnishes results very difficult of interpretation. Certain stocks, however, especially XII (B) and OF5, which have produced large trees associated with small crops, appear to have definitely induced low yields in the early history of the trees.

(*d*) *Precocity.*

For both varieties, precocity has been significantly affected by the rootstock and the order of stocks is almost identical for the two varieties. With Worcester Pearmain, IX appears as the most precocious stock whilst with the two varieties the vigorous stocks OF5, XII and B show the reverse character. Of the other stocks, II and IV are associated with relatively precocious fruiting and indeed, with Lane's Prince Albert, these two stocks together with VII do not differ significantly from IX.

The low position occupied by the very vigorous stocks OF5, Mall. XII and Mall. B is due both to the very light crops borne by these trees and to their relatively large size, whilst in the case of Mall. X poor fruiting is the dominant factor. Mall. I, which with Lane's Prince Albert is the most vigorous of the stocks examined, yielded a fairly heavy weight of fruit which compensated for the extra growth, so that the stock occupies an intermediate position. With IX the heavy crops borne, especially with the Worcester Pearmain, are accentuated by the small tree size.

(*e*) *Periodicity—Shoot Growth.*

This appears to be a factor of some importance in relation to flower bud formation (19). On the whole the data obtained confirm the observations of

Swarbrick made in earlier years. The date at which a pronounced drop in growth rate occurs seems to be related to the precocity of the trees. An early retardation, believed to favour carbohydrate accumulation, is associated with heavy cropping in the early years of the life of the trees. Under Long Ashton conditions, mid-July appears to be a critical period as far as initiation of flower bud primordia is concerned. Consequently the discussion is restricted to the data for this period. A consideration of relative as well as absolute growth rates was thought desirable, as trees which had made considerable growth early in the year and which consequently had a large leaf surface, might be assimilating sufficient to maintain a considerable growth rate and yet allow of carbohydrate accumulation.

Growth rates for the mid-July period appeared to be correlated with precocity. For both varieties correlation coefficients were extracted in order to express this relation numerically.

Mean values have been obtained exclusive and inclusive of stock Mall. IX and in the following values those including Mall. IX are given in parentheses.

Correlation between Relative Growth Rates and Precocity.

Worcester Pearmain.

Relative growth rate for period July 15th to 23rd.

$$r = -0{\cdot}6581 \pm 0{\cdot}1890 \quad (-0{\cdot}6718 \pm 0{\cdot}1939)$$

Lane's Prince Albert.

Relative growth rate for period July 6th to 18th.

$$r = -0{\cdot}5219 \pm 0{\cdot}2425 \quad (-0{\cdot}3589 \pm 0{\cdot}3080)$$

Essentially similar results are obtained when absolute instead of relative growth rates are considered.

RELATION OF CHEMICAL AND POMOLOGICAL DATA.

(*a*) *Shoot composition in relation to pomological grouping of the rootstocks.*

The pomological data relating to tree size and vigour to 1926 and 1928 respectively may be taken as giving a fairly accurate picture of the effects of the various rootstocks in promoting vegetative growth in the two varieties up to the present time. It has been pointed out that shoot measurements in 1931 indicated some change in the relative growth orders, mainly for the rootstocks No. V (both varieties) and No. VII (Lane's Prince Albert). These results, however, may be regarded as of a seasonal character since examination of the trees in 1934 shows that no substantial change of the old order has occurred. Accepting this as the position, we may discuss the chemical results in a general way in relation to the pomological data. Some detailed correlations on a statistical basis are presented in the following section.

The data for vigour show that in the case of Worcester Pearmain, Nos. XII, OF5, IV and I may be regarded as inducing strong growth, Nos. II, V and X as intermediate and Nos. VII and IX as producing dwarfing tendencies. With Lane's Prince Albert, Nos. I, OF5, B and II, and perhaps IV, produce strong growth, Nos. V and X are intermediate and Nos. VII and IX are again associated with pronounced dwarfing effects.

If these groupings are examined in relation to the results shown in Table III, it does not appear on the surface that the pomological characters show any close relationship to the various chemical factors. This is possibly due to the fact that such features as strong vegetative growth, dwarfing, precocity, etc., in the various cases are due to somewhat different causes. Nevertheless, there are several points of interest which require mention.

In the first place it is of interest to examine the results for No. IX rootstock. In this case the most interesting point is that there is only one case of a constituent which appears as *low* for this stock with either variety, viz. nitrogen for Worcester. On the other hand, values are on the high side for total ash, lime and ratio total ash/nitrogen for both varieties, potash and potash/nitrogen being also high for Worcester Pearmain and phosphate for Lane's Prince Albert. A low value for phosphate as reported by Colby (3) was not found.

It is apparent from the results that the characteristic dwarfing effect of this stock cannot be explained from the chemical data here available.

The second group of stocks for which it is of interest to follow the chemical results comprises Nos. II, V, VII and X. Trials have shown that these stocks may usually be classed as of intermediate vigour and are liable to give uncertain and variable results under varying soil conditions. No. II may perhaps be grouped in the strong group for Lane's Prince Albert in the present trials. In particular, Nos. II, V and VII have been shown to be susceptible to low potash conditions. Moreover, potash deficiency has been demonstrated by Wallace to be frequently influenced by nitrogen supply and thus it becomes of interest to examine the results for these stocks in Table III in the light of nitrogen and potash status and of secondary effects of low potash conditions. In making this examination, it must be remembered that the plot has been liberally manured with potash manures and hence drastic deficiency effects would not be expected and indeed have been absent throughout the experiment.

Potash deficiency is frequently associated with high content of dry matter and low total ash.

No. II shows high dry matter for both varieties as does No. V for Worcester Pearmain, whilst No. VII is in the high group for Lane's Prince Albert and in the low for Worcester Pearmain. For total nitrogen, Nos. II and V are high in both cases, whilst No. VII is high for Lane's Prince Albert only. It should be observed that No. X has a low nitrogen content in Lane's Prince Albert.

As regards total ash, No. II is low for both varieties, whilst No. V is low in Lane's Prince Albert. No. X is high for the latter variety.

For potash content, Nos. II and V are low in both cases and Nos. VII and X for Worcester Pearmain only.

In the values for the ratio, total ash/nitrogen, Nos. II and V are low for both varieties whilst No. X is low for Worcester and high for Lane's Prince Albert.

In the important ratio, potash/nitrogen, Nos. II and V are again low in both cases whilst No. VII is low for Lane's Prince Albert and No. X low for Worcester.

It will thus be seen that even under these favourable conditions of potash supply Nos. II and V stocks show considerable evidence of conditions suggesting low potash plants. The evidence in the other two cases is conflicting, especially for No. X, and does not provide a clear picture.

The three rootstocks, No. XII, B and OF5, have earned reputations as promoting vigorous vegetative growth and the present trial is no exception to this. It is thus of interest to follow the salient chemical points in relation to them. These are as follows:

No. XII (Worcester Pearmain only). Dry matter low; total ash high; lime high; magnesia high; ratio potash/nitrogen high.

No. B (Lane's Prince Albert only). Dry matter low; magnesia high; ratio potash/nitrogen high.

OF5. Total ash low (Lane's Prince Albert); lime and magnesia low (both varieties); potash high (Worcester Pearmain); phosphate low (Lane's Prince Albert); potash/nitrogen high (Worcester Pearmain).

The one point on which agreement occurs for all stocks (Worcester Pearmain only for OF5) is in the high ratio for potash/nitrogen. The low dry matter found in Nos. XII and B would be a condition likely to be associated with active vegetative growth.

Rootstocks I and IV are also of interest because of their vigour, No. I in the present trial especially as it heads the list for Lane's Prince Albert, and No. IV in view of the association of vigour with precocity. The salient features shown in the data for these stocks are as follows:

No. I. Total nitrogen low (Lane's Prince Albert); total ash high (both); lime high (Worcester Pearmain); magnesia high (Lane's Prince Albert);

potash high (Lane's Prince Albert); total ash/nitrogen high (Worcester Pearmain); potash/nitrogen low (Worcester Pearmain). This last value could not be obtained for Lane's Prince Albert owing to a missing value. The high potash value and possible low nitrogen value (two cases only) for Lane's Prince Albert are in agreement with the results for the other strong stocks but low potash/nitrogen value for Worcester is exceptional in a strong group.

No. IV. Total ash low (Worcester Pearmain); lime low (both); magnesia low (both); potash/nitrogen high (both). Again the potash/nitrogen value is high. The low value for lime requires note since the stock appears outstanding in this respect in this experiment.

Possible striking relationships between precocity and chemical features may be observed by arranging the stocks for each of the two varieties into three groups for precocity as shown below and examining the chemical results in Table III in relation to this grouping.

Group of high precocity, Nos. IX, II, IV; medium precocity, I, VII, V; low precocity, OF5, B, XII, X. When this is done, it is found that there is no agreement between the chemical features of the members of the respective precocity groups. The only case where all the rootstocks of a group show a common chemical feature is in the group of medium precocity where No. V (both varieties), No. I (Worcester Pearmain only), No. VII (Lane's Prince Albert only) all exhibit a low potash/nitrogen ratio. The results for this feature for the other two groups strongly suggest that this result is of no importance.

(*b*) *Statistical Correlations between Pomological Data and Chemical Constituents of Shoots.*

For the purpose of correlating the various pomological features of the trees with the shoot composition, the following tree characters were used.

(i.) Total wood growth to 1926.

(ii.) Mean growth of leader shoots in 1931.

(iii.) Growth rates of leader shoots for mid-July found in 1931.

(iv.) Relative growth rates of leader shoots for period mid-July 1931.

(v.) Precocity.

These characters were compared with the amounts of total nitrogen and ash constituents in the leaves, bark and wood of the terminal shoots by means of dot diagrams. Where there was any suggestion of a relationship, correlation coefficients were extracted with results as detailed below. Mall. IX was widely separated from the other stocks in growth characters and the values for r appended were obtained excluding stock IX.

Worcester Pearmain.

Nitrogen content of wood and growth of leader shoots—1931.

$+0{\cdot}6893 \pm 0{\cdot}1856$

Calcium content of leaves and growth of leader shoots—1931.*

$+0{\cdot}6579 \pm 0{\cdot}2359$

Potash content of bark and total growth to 1926.

$+0{\cdot}2020 \pm 0{\cdot}3390$

Phosphate content of wood and growth rates of leader shoots for period July 15th to 22nd.

$-0{\cdot}3035 \pm 0{\cdot}3210$

$\frac{P_2O_5}{N}$ for wood and growth rates July 15th to 22nd.

$-0{\cdot}7818 \pm 0{\cdot}1375$

$\frac{P_2O_5}{N}$ for wood and relative growth rates July 15th to 22nd.

$-0{\cdot}7700 \pm 0{\cdot}1536$

$\frac{P_2O_5}{N}$ for wood and precocity.

$+0{\cdot}8054 \pm 0{\cdot}1243$

Lane's Prince Albert.

Nitrogen content of wood and growth of leader shoots—1931.

$+0{\cdot}6433 \pm 0{\cdot}2231$

Calcium content of leaves and growth of leader shoots—1931.*

$+0{\cdot}6363 \pm 0{\cdot}2104$

Potash content of bark and total growth to 1926.

$+0{\cdot}7749 \pm 0{\cdot}1410$

Phosphate content of wood and growth rates of leader shoots for July 6th to 18th.

$-0{\cdot}3616 \pm 0{\cdot}3074$

$\frac{P_2O_5}{N}$ for wood and growth rates July 6th to 18th.

$-0{\cdot}6766 \pm 0{\cdot}1918$

$\frac{P_2O_5}{N}$ for wood and relative growth rates July 6th to 18th.

$-0{\cdot}2643 \pm 0{\cdot}3510$

$\frac{P_2O_5}{N}$ for wood and precocity.

$+0{\cdot}5741 \pm 0{\cdot}2316$

* For both varieties stock IV is in this respect widely separated from the other stocks.

The more interesting points which are evidenced in the correlation tables are as follows:

Only in one case is an entirely consistent significant result found for the two varieties, a significant negative correlation between the ratio $\frac{P_2O_5}{N}$ for wood and growth rates during the July period. In the case of Worcester Pearmain this ratio also shows a significant negative correlation with relative growth rate during July and a significant positive correlation with precocity.

It should be noted that P_2O_5 content of the wood is not significantly correlated with the July growth rates.

Two other correlations approach significance for both varieties: nitrogen content of wood and growth of leader shoots in 1931 show a significant positive correlation for Worcester Pearmain and the value is almost significant for Lane's Prince Albert; similarly calcium content of leaves and growth of leader shoots show a significant positive correlation for Lane's Prince Albert and an almost significant value for Worcester Pearmain.

Potash content of bark and total shoot growth to 1926 are positively correlated for Lane's Prince Albert but the correlation for Worcester Pearmain is not significant.

At the present stage it is not possible to foretell to what extent the correlations established in this experiment between the various chemical and pomological features will be found to be true with other varieties and with other conditions of growth.

(*c*) *Fruit Composition in relation to the Rootstocks.*

It has been mentioned previously in referring to Table II, that although significant groups of stocks occur in connection with most constituents, yet the numbers of groups are relatively few in each case. In spite of this, it has been considered worth while examining the main points shown in Table IV to see whether any prominent features of interest are apparent.

Fruits on No. IX stock are generally notable for large size, high colour and sweetness to taste, and in the present instance the latter two points are apparent, though as regards size, the data show that the size of the No. IX fruits has been inclined to be small rather than large. The chemical data show high values for dry matter and all sugars, and low values for potash.

In the case of No. VII, where dwarfing also occurs, nitrogen, reducing sugars and total sugars are high, and acid and phosphorus low.

No. V stock in many cases is associated with the production of poor quality fruit. The main features of the chemical results here are high sucrose and phosphorus, and low nitrogen, total sugars, ash and potash. The result for nitrogen is opposite from that in the shoots, but potash is again low.

The only other stocks which show marked tendencies in composition are the very strong stocks Nos. XII, B and OF5.

These all agree in showing high values for nitrogen, acid, ash and potash, and low amounts of reducing sugars. In addition, Nos. XII and B exhibit high phosphate whilst B and OF5 show low dry matter and total sugars. It may, of course, be possible in this group that these parallel tendencies are associated with the lighter crops which they have borne in these early years.

CONCLUSIONS.

In this investigation where the main object was to examine the effects produced by certain rootstocks, used with two varieties of apples, Worcester Pearmain and Lane's Prince Albert, in a field experiment, on various chemical features of the terminal shoots and fruits, the following conclusions were reached concerning the pomological and chemical data, the relationship between these and the influence of the rootstocks on the pomological and chemical characters.

A. Pomological Data.

Rootstocks produced significant effects on both varieties in the following cases:

a. Vigour as shown by total shoot growth from 1920 to 1926 and 1928 for Worcester Pearmain and from 1920 to 1926 for Lane's Prince Albert.

b. Seasonal growth of terminal shoots in 1931 and shoot growth to mid-July in that year.

c. The orders of the rootstocks under a and b did not entirely agree, mainly due to the special behaviour of trees on Nos. V and VII stocks in 1931, when they appeared to make abnormally strong shoot growth. No. VII, especially with Lane's Prince Albert, exhibited late growth during that season which resulted in different orders in the records to July and the end of the season respectively under b.

d. Periodicity of shoot growth as expressed by growth rates (cm. per day) and relative growth rates (growth per cm. per day) during periods in July 1931.

e. Total crops harvested to 1926 and 1928 respectively.

f. Precocity as expressed by weight of fruit produced per 100 metres of shoot growth to 1926. There were five significant groups for Worcester Pearmain and three for Lane's Prince Albert, and the orders of the stocks for the two varieties were similar. Precocity showed a significant negative correlation with growth rates of leader shoots for the mid-July period for Worcester Pearmain and a similar correlation, almost significant, for Lane's Prince Albert.

B. CHEMICAL DATA.

Shoots.

a. A negative correlation was established between calcium and potash as percentages of total ash in leaves, bark and wood.

b. Correlations for the respective elements in regard to their contents in the different portions of the shoots—leaves, bark and wood—were found as under:

1. A positive correlation for nitrogen between all three pairs of tissues.
2. A positive correlation for potash between leaves and wood, and leaves and bark.
3. A positive correlation for magnesium between leaves and bark, and for calcium between bark and wood.

c. Using Fisher's Z test, and taking the 5 per cent. point as a measure of significance, significant stock effects appeared for total nitrogen in leaves, total ash in bark, potash in wood, phosphate in bark and wood, and calcium oxide in leaves. The values for total nitrogen in wood and bark, potash in leaves, phosphate in leaves and calcium oxide in bark also approached the significant point.

Fruits.

d. Significant effects due to rootstocks were established for all chemical constituents with the exception of reducing sugars, and potash in ash, for Worcester Pearmain, and sucrose and total ash for Lane's Prince Albert. The effects were not of great magnitude, since for each constituent the number of rootstock groups was small.

C. RELATIONSHIPS BETWEEN CHEMICAL AND POMOLOGICAL DATA.

a. The following correlations were established.

Negative correlation between $\frac{P_2O_5}{N}$ in wood and growth rates of terminal shoots during July 1931 (both varieties).

Negative correlation between $\frac{P_2O_5}{N}$ in wood and relative growth rates in July 1931 (Worcester Pearmain).

Positive correlation between $\frac{P_2O_5}{N}$ in wood and precocity.

Positive correlation between N in wood and growth of leader shoots in 1931 (Worcester Pearmain): also a positive correlation, nearly significant, for Lane's Prince Albert.

Positive correlation between Ca content of leaves and growth of leader shoots in 1931 (Lane's Prince Albert): also nearly significant for Worcester Pearmain.

Positive correlation between potash in bark and total shoot growth to 1926 (Lane's Prince Albert).

D. SPECIAL FEATURES OF ROOTSTOCK EFFECT IN RELATION TO CHEMICAL CHARACTERS.

a. The characteristic dwarfing effect of Mall. IX rootstock cannot be explained on a basis of any of the chemical features examined.

b. Although the trees were grown under relatively favourable conditions of potash supply, the chemical data on the rootstocks Mall. V and Mall. II showed considerable evidence of low potash conditions. Trees on Mall. VII rootstock also provided some evidence in this direction but those on Mall. X did not give a clear picture in respect of this.

c. The data for trees on the rootstocks promoting great vigour, viz. Mall. XII, Mall. B and OF5, only agreed completely in respect of one character in shoot composition, a high $\frac{K}{N}$ ratio.

d. The main points relating to fruit composition in relation to individual rootstocks were as follows: Fruits on Mall. IX trees showed high values for dry matter and all sugars; those on Mall. V contained low total sugars, ash and potash; the fruits from the vigorous stocks Nos. Mall. XII, Mall. B and OF5 all showed high values for nitrogen, acid, ash and potash, and low values for reducing sugars.

SUMMARY.

An investigation is described in which an attempt was made to ascertain the extent to which effects produced by a number of rootstocks in combination with two scion varieties of apples were related to certain chemical features of the terminal shoots and fruits.

The trees utilized in the work comprised the two scion varieties Worcester Pearmain and Lane's Prince Albert, worked on vegetatively propagated rootstocks in each case.

For one section of the investigations, relating to shoots, only nine stocks were considered for each variety, but for the other section, concerning fruits, all twelve stocks were included for each scion.

Since planting in 1920 until 1928, detailed pomological records on the trees had been obtained, using the individual tree as the unit, and these records provided the bulk of the pomological data considered. They related to annual

total shoot growth, and total weights of crops produced. From these it was possible to calculate values for precocity, defined as total weight of fruit produced per 100 metres of shoot growth to 1926.

In addition, the following records were collected during 1931, as a measure of the growth conditions in that season, when the shoot samples were taken for chemical analysis: seasonal shoot elongation and shoot elongation to mid-July; growth rates of shoots for periods in July and relative growth rates for the same periods.

The chemical determinations were carried out on terminal shoots collected during July 1931 and on fruits from crops of three seasons within the period 1927-31.

The chemical constituents considered were as follows:

Shoots: total nitrogen, dry matter, total ash, and the ash constituents calcium, magnesium, potassium and phosphorus.

Fruits: in addition to the above constituents, determinations were made of reducing sugars, sucrose, total sugars and titratable acidity.

The pomological and chemical data were treated statistically as far as possible and various correlations determined.

It was shown that the rootstocks had produced significant effects on both pomological and chemical characters, whilst certain correlations were also established between various chemical constituents, and between chemical and pomological features.

Certain special features of rootstock effect were examined in relation to the chemical data. It was not found possible to explain the dwarfing effect of Malling No. IX stock on a basis of any chemical feature. It was shown that the rootstocks Mall. II, Mall. V, known to be susceptible to potash deficiency, had many of the characters of low potash plants, even under conditions of favourable potash supply. Rootstocks promoting great vigour only showed agreement in respect of one character in the shoots, a high $\frac{K}{N}$ ratio.

The results show that although the rootstocks produced significant differences as regards certain chemical constituents in the terminal shoots and the fruits of the trees of both varieties, many of the outstanding rootstock effects could not be explained on the basis of the chemical characters examined.

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BIBLIOGRAPHY.

(1) *Brown, J. W.* Chemical Studies on the Physiology of Apples. V. Methods of Ash Analysis and the Effect of Environment on the Mineral Constitution of the Apple. Ann. Bot., Vol. XL, 1926.

(2) *Chibnall, A. C.* Diurnal Variations on the Total Nitrogen Content of Foliage Leaves. Ann. Bot., Vol. XXXVII, 1923.

(3) *Colby, H. L.* Stock-Scion Chemistry in Apples. Abstracts papers presented before 10th Ann. Meeting of Amer. Soc. Plant Physiol, Dec. 1933.

(4) *Daniel, L.* Recherches Morphologiques et Physiologiques sur la Greffe. Revue Generale de Botanique, Vol. 6, 1894.

(5) ———. Réaction antagonistique et rôle du bourrelet chez les plantes greffées. Compt. Rend., Vol. 170, 1920.

(6) *Daniel, L.* and *Thomas, V.* Sur l'utilisation des principés mineraux par les plantes greffées. Soc. Scient et Medicale de l'ouest, Bull. 11, 1902.

(7) *Fisher, R. A.* Statistical Methods for Research Workers. London, 1930.

(8) *Haas, A. R. C.* and *Halma, F. F.* Chemical Relationship between Scion and Stock in Citrus. Plant Physiol., Vol. 4, p. 113, 1929.

(9) *Hatton, R. G.* Masters Memorial Lectures, 1929. Stock/Scion Relationships. Journ. Royal Hort. Soc., Vol. LV, Part 2, p. 169, 1930.

(10) *Javillier, M.* Sur la migration des alcaloïdes dans les greffes de Solanées sur Solanées. Compt. Rend., Vol. 150, 1910.

(11) *Knight, R. C.* Water Relation of Stocks and Scions, Progress Report. East Malling Res. Sta. Ann. Rept., Part II, p. 103, 1924.

(12) *Lane, J. H.* and *Eynon, L.* Determination of Reducing Sugars by means of Fehling's solution with Methylene Blue as Internal Indicator. Journ. Soc. Chem. Ind., Vol. XLII (Trans.), 1923.

(13) *Mason, T. G.* and *Maskell, E. J.* Studies on Transport of Carbohydrates in the Cotton Plant. I. Study in Diurnal Variation in Carbohydrates of Leaf, Bark and Wood and of the Effects of Ringing. Ann. Bot., Vol. LXII, 1928.

(14) *Meyer* and *Schmidt.* Uber die gegenseitige Beeinflussing der Symbionten heteroplastischer Transplantationen mit besonderer Berucksichtungung der Wanderung der Alkaloide durch die Pfropfstellen. Flora oder Allgemeine Botanische Zeitung, 1910.

(15) *Mulay, A. S.* Statistical Study of the Total Nitrogen in Bartlett Pear Shoots. Plant Physiol., Vol. 6, p. 333, 1931.

(16) *Rivière, G.* and *Bailhache, M.* Contribution à la Physiologie de la Greffe. Influence du porte-greffe sur le Greffon. Compt. Rend., Vol. 124, 1897.

(17) *Roach, W. A.* Chemistry of the Rootstock-Scion Effect. East Malling Research Station Supplement, p. 101, 1928-9-30.

(18) *Strasburger, E.* Ueber Verendlungen und Ueber Verwachsungen und deren Folgen. 1884.

(19) *Swarbrick, T.* Factors Governing Fruit Bud Formation. VIII. The Seasonal Elongation Growth of Apple Varieties on some Vegetative Rootstocks; and its possible relation to Fruit Bud Formation. Journ. Pom. & Hort. Sci., Vol. VII, p. 100, 1928.

(20) *Wallace, T.* Factors Influencing the Storage Qualities of Fruits. Proc. 1st Imper. Hort. Conf., Part III, p. 9, London, 1930.

(21) *Winkler, Hans.* Researches on Graft Hybrids. Jena, 1912.

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A STUDY OF THE VARIATIONS IN LEAF SHAPE AND PETIOLE LENGTH IN SEEDLINGS OF "PARADISE" APPLES

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WHILE the cytology of varieties of the genus Pomoideae have been the subject of much attention during recent years, considerable obscurity still surrounds the purely genetical question of the inheritance of characters in varieties of the apple and pear. The elucidation of the subject is unquestionably attended by serious difficulty and accessions to our knowledge must of necessity be slow.

The work of Crane and Lawrence (1) in this country and of Wellington (2) and Lantz (3, 4) in America, has given support to the view that the inheritance of many of the characters in the apple is determined by the interaction of a greater number than a single pair of genes. Such a view corresponds closely with Darlington and Moffett's (5) interpretation of chromosome association in the apple and is largely based on the fact that the variation of most of the characters so far studied in apple seedlings appears to the observer to be less specific and discontinuous than would be expected on the basis of simple disomic inheritance.

A considerable programme of breeding work, of a purely practical kind, is being carried out jointly by the John Innes Horticultural Institution and the East Malling Research Station with the object of evolving new varieties of apple rootstock embodying improvements upon the varieties at present in commercial use. Much attention has been paid of late years to the part which the rootstock plays in influencing the vigour and fruitfulness of apple trees. In particular, the investigations of Hatton (6, 7) on the group of rootstocks now collectively known as "Paradise" have brought about a considerable modification and improvement in commercial fruit growing practice.

All those varieties of "Paradise" so far investigated by the workers at Merton have been found to be diploids ($2n=34$) as to their chromosome constitution. Their origin is somewhat obscure but Bunyard (8) cites Molon as placing certain of them as sub-varieties of *Malus pumila*. Of the varieties used in the crosses dealt with in this paper Doucin (Malling No. II) and Jaune de Metz (Malling No. IX) have been known on the Continent for many years, while River's Nonsuch (Malling No. VI) was introduced by Messrs. Rivers, the English nurserymen, in the middle of last century. While these varieties can be intercrossed very readily, a long period, upwards of twenty years, must elapse before a full idea of the economic potentialities of the seedlings can be obtained. The methods of testing and elimination employed are not relevant to the present study and may be found elsewhere (9). The attention of those responsible for this work has been directed towards the idea of finding a method by which those seedlings likely to make valuable new rootstocks could be selected at an earlier stage than is now possible. A study of their comparative physiology or biochemistry afforded possible avenues of approach. At the same time, since it could scarcely be doubted that such characters of the seedling as the capacity to regulate the vigour and fruitfulness of a scion variety, in vegetative union with it, was the expression of the interaction of genetic factors, it was thought that such factors might be linked in inheritance with factors controlling easily recognizable morphological characters manifesting themselves early in the life of the seedling. This would enable, at the least, a preliminary discarding of worthless individuals to be made, during the first few years.

During the years 1931-3, a careful study was made of about 1,000 seedlings, the results of crosses between Malling No. IX and Malling Nos. I, II, IV, V, VI, VII, XII, XIII, XV and XVI. The twenty-eight characters studied covered fairly comprehensively the morphology of the summer and winter shoots and of the root systems. Details may be found elsewhere (10). The findings were in agreement with those of other workers who have studied variation in apple seedlings. Variation was in most cases more or less continuous between extremes and little evidence of segregation into distinct classes could be obtained. Since, however, most of the characters studied were such that only an approximate classification could be adopted, it was thought worth while to study in greater detail two characters of which comparative measurements could be obtained. The two characters chosen were the comparative breadth of the lamina of the leaf and the length of the leaf petioles. There are considerable differences between the various varieties of "Paradise" apple rootstocks with regard to both these characters. The leaves were selected from the shoots which arose from the stools,* leaves from the same portion of the shoots being selected in each case. Ten leaves were measured from each of 525 seedlings and for purposes of comparison 100 leaves, taken at random from about 400 stools of each of the parent varieties, were measured.

I. Leaf Shape.

In dealing with the figures presented in Table I it will be best to consider first the differences in leaf shape shown by the clonal races of rootstocks used as parents in these experiments. As may be seen, variability within the hundred leaves, taken at random, was not high, ranging between 6 and 10·5 per cent.

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* These rootstocks, when vegetatively propagated, are cut down and mounded up with soil. Shoots arise each season from basal buds below soil level and comprise new individuals when severed from the stool.

It is possible to group these varieties into three classes: (a) No. VI with a $\frac{\text{Breadth} \times 100}{\text{Length}}$ figure of 59; (b) Nos. I, II, V and IX with a $\frac{\text{Breadth} \times 100}{\text{Length}}$ figure of approximately 66; and (c) Nos. IV and VII with a $\frac{\text{Breadth} \times 100}{\text{Length}}$ figure of about 78. These three classes were found to be significantly different when tested by the methods of modern statistical analysis.* When the rootstocks Nos. I, II and V (Class B) were crossed with No. IX the $\frac{\text{Breadth} \times 100}{\text{Length}}$ figure obtained by averaging the six families was 65·2, almost identical with that of the parents; when No. VI (Class A) was crossed with No. IX (Class B) the figure obtained was 65·5 which is almost identical with that obtained when No. IX was crossed with the varieties in Class B. Finally, when No. IX was crossed with Nos. IV and VII (Class C) the $\frac{\text{Breadth} \times 100}{\text{Length}}$ figure obtained by averaging the three families was 67·8. Thus, the seedlings obtained from crossing Nos. IV and VII with No. IX, while they have, on the average, slightly broader leaves than was the case when the remaining four varieties were crossed with No. IX, have leaves considerably more narrow than those borne by the parent varieties IV and VII. Tested statistically, it was found that the difference in leaf shape between the three families IV and IX, IX and IV, VII and IX and the remaining families is significant and, futhermore, that the difference between the IV and IX seedlings and the VII and IX seedlings is statistically significant. As may be seen from the coefficients of variability given in Table I, variability in the seedlings was little greater than that found in the parent varieties and there is no evidence that variability was markedly higher in some families than in others. From the figures given in Table II it will be seen that there was no apparent discontinuity whatever in the seedling families.

In discussing the data set out above, three possibilities occur to one, each of which will partially fit the facts:

(a) That all these varieties of rootstock are genetically similar with respect to this character but that No. VI on the one hand and Nos. IV and VII on the other represent the extremes of variation in a single phenotype.

(b) That No. IX is homozygous and dominant for this character.

(c) That a number of polymeric genes are involved in the expression of this character.

It is not difficult to find objections to each of these hypotheses. It seems improbable that varieties which show a statistically significant difference in respect of a character are yet genetically identical and, moreover, that seedling

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* The writer is indebted to Miss J. Edgar for her very careful statistical analysis of much of the data presented in this paper.

TABLE I.

	Number of leaves.	Breadth of leaf expressed as percentage of length of lamina.	Coefficient of Variability %		Number of Seedlings.	Number of Leaves.	Breadth of leaf expressed as percentage of length of lamina.	Coefficient of Variability.	
								Between Seedlings %	Between Leaves %
Malling No. VI ..	100	59·02	8·95	No. VI × No. IX ..	74	740	65·48	7·07	*9·75
Malling No. I ..	100	65·76	7·75	No. I × No. IX ..	66	660	68·60	6·62	8·43
				No. IX × No. I ..	73	730	66·75		9·43
Malling No. II ..	100	65·04	6·27	No. II × No. IX ..	39	390	64·05	7·94	9·69
				No. IX × No. II ..	7	70	62·13		9·31
Malling No. V ..	100	64·90	10·53	No. V × No. IX ..	49	490	64·50	6·61	10·46
Malling No. IX ..	100	67·57	5·99	No. IX × No. V ..	16	160	65·06		8·00
Malling No. IV ..	100	78·02	7·43	No. IV × No. IX ..	97	970	67·71	6·04	9·20
				No. IX × No. IV ..	52	520	69·46	6·80	9·55
Malling No. VII	100	78·16	6·71	No. VII × No. IX	52	520	66·23	10·64	8·13

	Number of Leaves.	Length of Petiole expressed as percentage of length of lamina.	Coefficient of Variability %		Number of Seedlings.	Number of Leaves.	Length of Petiole expressed as percentage of length of lamina.	Coefficient of Variability.	
								Between Seedlings %	Between Leaves %
Malling No. I ..	100	25·49	14·23	No. I × No. IX ..	66	660	26·25	13·57	*17·59
				No. IX × No. I ..	73	730	26·38		19·18
Malling No. V ..	100	26·22	17·21	No. V × No. IX ..	49	490	26·42	12·71	17·16
Malling No. IX ..	100	28·87	12·13	No. IX × No. V ..	16	160	27·06		16·72
Malling No. VI ..	100	29·97	16·63	No. VI × No. IX ..	74	740	25·55	12·15	14·09
Malling No. II ..	100	30·90	12·36	No. II × No. IX ..	39	390	25·15	13·30	17·77
				No. IX × No. II ..	7	70	23·54		14·47
Malling No. IV ..	100	38·78	17·37	No. IV × No. IX ..	97	970	28·94	14·94	18·85
				No. IX × No. IV ..	52	520	30·52	14·00	17·18
Malling No. VII	100	35·40	12·52	No. VII × No. IX	52	520	30·20	12·47	15·43

* Calculated on 100 leaves taken at random in each case.

families raised from crosses between such varieties should differ significantly amongst themselves. If No. IX is assumed to be dominant for leaf shape it is not easy to account for the fact that families IV × IX, IX × IV, and VII × IX were broader, on the average, than the remaining families. The results, however, point to the action of polymeric factors, and indicate that a number of such factors are carried by all the rootstocks used as parents in these experiments and the variation and lack of discontinuity in the families supports such a view.

II. Length of the Leaf Petiole.

On the lower part of Table I will be found the average length of the leaf petioles of the clonal rootstocks and the seedlings. They have been expressed as a percentage of the length of the leaf lamina to render them comparable. As may be seen the clonal races of rootstocks may be divided into three classes, (a) Nos. I and V with petioles averaging about 26 per cent. of the leaf blade, (b) Nos. II, VI and IX with petioles about 30 per cent. of the leaf blade, and (c) Nos. IV and VII with petioles around 37 per cent. of the leaf blade. The difference between these three classes is statistically significant and it should be added that there is a significant difference between Nos. IV and VII. As may be seen, the coefficient of variability was high, round about 15 per cent. of the mean, and is, in fact, almost exactly twice as great as that obtained when the relative leaf breadth was studied. With regard to the figures for the seedling families when No. IX was crossed with Nos. I and V, the petioles averaged about 26½ per cent. of the length of the lamina; where No. IX was crossed with Nos. II and VI the average was about 24½ per cent. of the lamina length. Finally, when No. IX was crossed with Nos. IV and VII the petiole length averaged about 30 per cent. of the length of the lamina. The three families, IV × IX, IX × IV, and VII × IX, when analysed statistically were found to have significantly longer petioles than the remaining families but there was no significant difference between them. It will be noticed that the variability in these families is little greater than that found in the clonal races and, from Table III, that there is no evidence of discontinuity in the inheritance of this character in the seedlings.

As with the character previously dealt with, it does not seem very easy to explain the inheritance of leaf petiole length on a simple Mendelian basis. The fact that there is no apparent discontinuity does not, of course, preclude the possibility of segregation in the seedlings, but the fact that the difference in variability between the parent varieties and seedlings is so small, makes it seem unlikely. The fact that in every case the petioles of the seedlings were, on the average, shorter than those of the two parents, taken together, may possibly be significant. Without more data it is probably not possible to say what was the nature of the inheritance involved.

TABLE II.

Frequency Table: Breadth of Leaf expressed as Percentage of Length of Leaf.

	50	52	54	56	58	60	62	64	66	68	70	72	74	76	78	80	82	84	86	88	90	92	94	96
Malling No. I ..	—	—	1	1	2	15	9	19	14	11	12	10	2	2	1	1	—	—	—	—	—	—	—	—
Malling No. II ..	—	1	—	1	4	9	19	16	19	16	7	4	4	—	—	—	—	—	—	—	—	—	—	—
Malling No. IV ..	—	—	—	—	—	—	—	1	2	5	—	10	10	18	12	17	13	—	4	3	3	1	1	—
Malling No. V ..	3	2	4	6	3	13	6	9	14	9	12	5	7	4	3	—	—	—	—	—	—	—	—	—
Malling No. VI ..	4	4	14	16	16	14	14	5	7	3	1	—	—	—	2	—	—	—	—	—	—	—	—	—
Malling No. VII ..	—	—	—	—	—	1	—	2	1	1	3	11	17	6	20	13	9	9	3	3	—	1	—	1
Malling No. IX ..	—	—	—	—	3	3	4	18	19	20	16	7	8	2	—	—	—	—	—	—	—	—	—	—
I×IX	—	—	—	—	—	2	6	5	11	12	14	8	4	1	—	1	1	1	—	—	—	—	—	—
IX×I	—	—	—	2	2	3	6	11	10	13	15	8	3	—	—	—	—	—	—	—	—	—	—	—
II×IX	1	—	—	3	4	1	4	6	11	5	1	3	—	—	—	—	—	—	—	—	—	—	—	—
IX×II	—	—	1	2	—	1	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
IV×IX	—	—	—	—	2	1	9	6	8	12	19	15	11	7	4	2	1	—	—	—	—	—		—
IX×IV	—	—	—	—	—	1	4	5	6	10	8	8	2	4	3	1	—	—	—	—	—	—	—	—
V×IX	—	—	1	2	1	5	9	11	8	4	5	2	—	1	—	—	—	—	—	—	—	—	—	—
IX×V	—	—	—	—	2	1	2	2	1	6	2	—	—	—	—	—	—	—	—	—	—	—	—	—
VI×IX	—	—	—	3	3	6	9	13	17	7	3	10	2	—	1	—	—	—	—	—	—	—	—	—
VII×IX	—	—	1	—	2	5	6	11	3	10	5	4	2	1	2	—	—	—	—	—	—	—	—	—

TABLE III.

Frequency Table: Length of Petiole expressed as Percentage of Length of Lamina.

	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58
Malling No. I	—	4	11	8	22	25	15	8	4	2	1	—	—	—	—	—	—	—	—	—	—	—
Malling No. II	—	—	—	—	4	13	19	18	21	8	12	4	—	1	—	—	—	—	—	—	—	—
Malling No. IV	—	—	1	—	1	1	4	6	6	9	11	12	15	11	5	9	3	1	3	1	1	—
Malling No. V	1	5	8	8	18	16	22	7	8	6	—	—	—	1	—	—	—	—	—	—	—	—
Malling No. VI	—	2	2	3	10	11	14	21	11	9	7	8	1	—	—	1	—	—	—	—	—	—
Malling No. VII	—	—	—	—	1	2	5	7	16	18	18	11	8	9	5	—	—	—	—	—	—	—
Malling No. IX	—	—	1	3	13	12	24	22	18	3	1	3	—	—	—	—	—	—	—	—	—	—
I×IX	—	—	4	7	15	9	13	7	4	2	1	—	—	—	—	—	—	—	—	—	—	—
IX×I	—	2	2	7	13	20	13	9	4	3	—	—	—	—	—	—	—	—	—	—	—	—
II×IX	—	—	3	10	8	6	8	2	2	—	—	—	—	—	—	—	—	—	—	—	—	—
IX×II	—	1	1	—	3	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
IV×IX	—	—	3	6	10	12	23	19	3	12	5	3	1	—	—	—	—	—	—	—	—	—
IX×IV	—	—	—	1	3	6	9	11	11	5	4	—	—	1	1	—	—	—	—	—	—	—
V×IX	—	1	3	5	8	11	7	9	4	1	—	—	—	—	—	—	—	—	—	—	—	—
IX×V	—	—	—	2	3	2	5	3	—	1	—	—	—	—	—	—	—	—	—	—	—	—
VI×IX	—	1	5	11	16	18	14	6	2	1	—	—	—	—	—	—	—	—	—	—	—	—
VII×IX	—	—	1	1	2	8	5	14	9	8	3	—	1	—	—	—	—	—	—	—	—	—

It cannot be pretended that it has been possible to arrive at any satisfactory conclusion as to the nature of the inheritance of these two characters although the data provides some interesting indications. The material, raised primarily for other purposes, was far from ideal for such an investigation. The fact that No. IX was a parent common to all the families makes analysis unsatisfactory and crosses between other varieties might have provided valuable additional information. On the other hand, the known complexity of the chromosome constitution of the apple may mean that only studies carefully undertaken on the most comprehensive scale can hope to succeed in elucidating the genetic inter-relationships of apple varieties.

SUMMARY.

Measurements of the breadth of the leaves and of the length of the leaf petioles of 525 seedlings from crosses between varieties of the "Paradise" apple rootstocks are summarized and discussed.

REFERENCES.

(1) *Crane, M. B.* and *Lawrence, W. J. C.* Genetical Studies in Cultivated Apples. Journ. of Genet., **28**, 2.

(2) *Wellington, R.* An Experiment in Breeding Apples. Tech. Bull. 160, New York Agric. Expt. Sta.

(3) *Lantz, H. L.* Apple Breeding—A Study of Jonathan Crosses. Iowa Agric. Expt. Sta. Res. Bull. 116.

(4) ———. Some Observations on the Effect of Inbreeding on the Vigour of Apple Seedlings. Proc. Amer. Soc. Hort. Sci., **22**, 115-23.

(5) *Darlington, C. D.* and *Moffett, A. A.* Primary and Secondary Chromosome Balance in Pyrus. Journ. of Genet., **22**, 2.

(6) *Hatton, R. G.* Paradise Apple Rootstocks. Journ. Roy. Hort. Soc., **42**.

(7) ———. The Relationship between the Scion and Rootstock with Special Reference to the Tree Fruits. Masters Memorial Lecture. Journ. Roy. Hort. Soc., **55**.

(8) *Bunyard, E. A.* The History of the Paradise Stocks. Journ. Pom & Hort. Sci., **1**.

(9) *Tydeman, H. M.* The Testing of New Varieties of Apple Rootstock. East Malling Res. Sta. Ann. Rept. for 1933 (May 1934).

(10) ———. Breeding Experiments with Paradise Apple Rootstocks. Journ. Pom. & Hort. Sci., **11**, 3.

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ON THE TRANSMISSION OF THE STRAWBERRY VIRUS "YELLOW-EDGE" DISEASE BY THE STRAWBERRY APHIS, TOGETHER WITH NOTES ON THE STRAWBERRY TARSONEMID MITE

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INTRODUCTION.

LATE in 1930 an investigation of a suspected virus disease of the variety Royal Sovereign was commenced (1, 3).

The constant association of the Strawberry Tarsonemid Mite (*Tarsonemus fragariae* Zimm.) with diseased plants suggested that it might be a vector of virus, and an initial experiment to test this possibility was carried out in 1931 in an insect-proof greenhouse. It was soon discovered however, that, under such conditions, it was almost impossible to keep the plants growing vigorously, and in addition, the mite population multiplied so rapidly that many of the plants were killed, while the remainder were so unhealthy that if symptoms of virus disease had been present they would have been completely masked. Thus, finally, it was decided to abandon all experiments under such conditions.

In the meanwhile Harris (1) in 1931 had devised a grafting technique whereby he was able in 1932 to prove the presence of a virus disease in the variety Royal Sovereign (2).

Once this disease could be diagnosed in the field it was noticed that many of these diseased plants were infested with insects and mites, which naturally suggested that one or more of these animals might function as a vector. This suggestion was strengthened by the fact that certain species of aphides were already known to be vectors of virus diseases of strawberry in the United States of America (5, 6).

1932-3 EXPERIMENT.

The first field scale experiment was laid down in October 1932. In this trial one healthy clone of Royal Sovereign plants was used. It consisted of 180 plants. By this time it had been demonstrated in another experiment that insects and mites could be eradicated from strawberry plants by immersing them in warm water for a given period at a certain temperature (4). All the plants used in this experiment were subjected to the warm water treatment before they were planted, and were thus practically free from mite and insect infestation. After treatment the plants were potted up into seven inch pots, and were plunged in rows, in six blocks, on a site adjoining the insect-proof greenhouse.

In this trial it was proposed to determine whether the Strawberry Aphis (*Capitophorus fragariae* Theo.) and the Strawberry Tarsonemid Mite (*Tarsonemus fragariae* Zimm.) were vectors of the virus disease. These animals were selected because they proved to be the ones most frequently observed on virus plants.

The inoculations with aphides and mites bred from both diseased and healthy plants were commenced at the beginning of July and were continued until the latter part of August.

Although no final conclusion could be drawn from the results of this experiment, they were sufficiently suggestive to warrant a repetition on a more extensive scale. Further, valuable information had been obtained in regard to technique, methods of breeding insects and mites required for inoculation purposes, and more particularly on ways of keeping the check plants free from attack. The results of the above trial can be summarized briefly as follows:

(1) Two of the plants inoculated with mites-from-diseased plants produced symptoms suggestive of virus disease.

(2) One of the plants inoculated with mites-from-healthy plants produced symptoms suggestive of virus disease.

(3) One of the control plants showed typical virus symptoms.

(4) Three of the plants inoculated with aphides-from-healthy plants produced virus symptoms.

(5) Four of the plants inoculated with aphides-from-diseased plants showed typical virus symptoms.

At the end of September 1933 all the plants in the experiment were once more subjected to the warm water treatment (the technique of which had been considerably improved by this time), which removed all mites and insects from the plants, and allowed some interesting observations to be made in the following year. Both lots of mite infested plants grew away in a normal manner, in fact they could not be separated from the control plants. Thus, it was proved that when the mite factor had been removed, the plants functioned quite normally. Virus plants were not noted in either of the mite series during the spring, summer or autumn of 1934. The suspicious symptoms noted on three plants in the autumn of 1933 were evidently the result of the very heavy mite infestation. It is almost, if not quite, impossible to diagnose virus on a severely mite infested plant, because of the masking effect caused by the mite attack. Even, therefore, if the mite does not prove to be a vector of virus, it will still be essential to eradicate it from the field, first because of the direct injury it causes to the plant, and second because of its masking the symptoms of the virus and consequently making roguing for virus disease impossible.

The seven plants in the aphis series which exhibited virus symptoms in 1933 again showed typical symptoms in the spring and autumn of 1934. One control plant also showed typical symptoms as it did in the previous year.

1933-4 EXPERIMENT.

In the autumn of 1932 a number of Royal Sovereign plants which had been subjects of the grafting experiment already referred to (2) and had thus been shown to be virus free, were warm water treated and laid down for runner propagation. These plants and their progeny were sprayed at weekly intervals as a matter of routine. During the first week of October 1933, two hundred runners were selected from one of these healthy clones, all being as far as possible equal in size and vigour. They were then subjected to the warm water treatment which has been fully described elsewhere (4). They were next transferred to seven inch pots, and were plunged in the soil in rows in six bays. Each of the six bays consisted of thirty plants. A complete plan of the lay-out of the plot is given on page 42. It will be seen that the two control bays are in the centre of the plot, while the bays where the aphis and mite inoculations were made are on the left and right respectively.

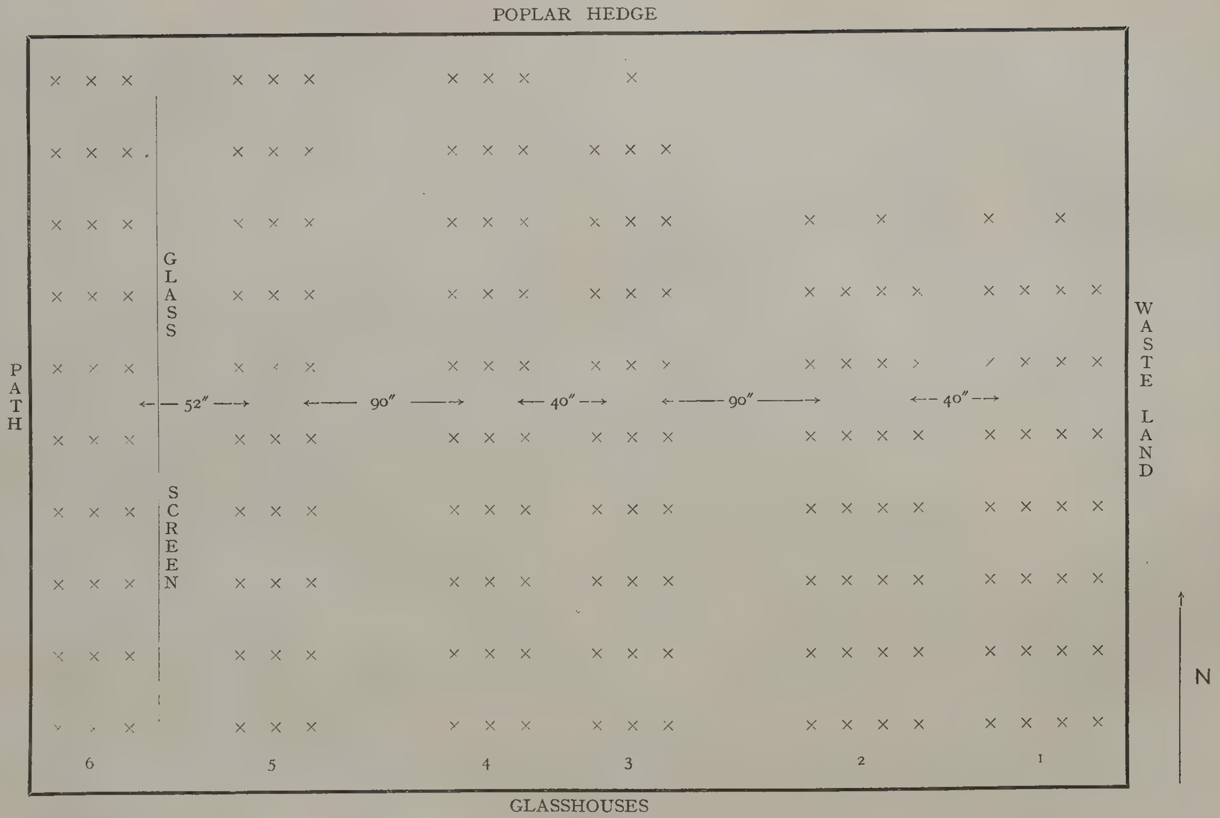
TREATMENT OF PLOTS.

In view of the fact that the plants were growing under natural conditions, and that no effort was made to enclose them in cages or muslin bags, rigorous precautions had to be adopted to keep off stray insects and mites which might be expected to settle upon them.

All the plants had been warm water treated in October 1933 ; they were thus all clear at the start, a great advance on the earlier investigations. Early in March 1934, as soon as new growth was beginning, all the plants were sprayed with a solution of lime-sulphur and nicotine. This routine spraying was continued on all the bays at weekly intervals until the end of May. After this date it was not possible to spray some of the bays because the direct inoculations were due to commence. The control bays were sprayed all through the season, however ; these plants received twenty-seven applications. The spray formula was modified from time to time, but the formula generally used was as follows: ¼ fl. oz. nicotine ; +5·7 grs. sulphonated lorol ; +8 fl. ozs. lime-sulphur ; in 2½ galls. water.

Since it was well known that a nicotine spray was not toxic to the Strawberry Tarsonemid Mite, it proved possible to spray the mite bays from time to time, even while the artificial inoculations were being made.

Plan of Lay-out of Experiment.


POPLAR HEDGE
PATH
GLASS SCREEN
WASTE LAND
GLASSHOUSES
N
52″
90″
40″
90″
40″
6
5
4
3
2
1

Key:

1. Inoculated with mites-from-virus-plants.
2. Inoculated with mites-from-healthy-plants.
3. Controls (first series).
4. Controls (second series).
5. Inoculated with aphis-from-healthy-plants.
6. Inoculated with aphis-from-virus-plants.

× = plants.

SOURCE OF APHIDS AND MITES.

All the strawberry aphis (*Capitophorus fragariae* Theo.) material used for making the direct inoculations was obtained from one source. Six apterous viviparous females were collected from a healthy Royal Sovereign plant in October 1933, and were transferred immediately to a healthy Royal Sovereign plant growing in a pot (and actually belonging to the same clone as that selected for the main trial). The plant was kept in a warm greenhouse. Under such conditions the aphides began to breed in January. Now, as soon as the nymphs were produced they were removed to six more plants of the same clone, growing in the same house. The aphides were allowed to breed freely on these plants. At the end of January the original plant inoculated was destroyed, since the aphis colonies on the other six clonal plants were now well established.

At the same time, in an adjoining greenhouse, a number of plants suffering from virus disease were being raised in pots. Now these plants were actually the progeny of the original virus material used by Harris in his grafting experiments. They were inoculated with a number of aphides from the healthy clonal plants in the next house, and a very strong colony was soon established. Thus, by the end of May, there proved to be an abundance of aphides on both healthy and diseased plants.

The mite material was procured and bred in a similar manner to the aphis material. In order not to complicate the problem unduly, the mites were bred on the same series of plants as the aphides. Thus six healthy, clonal Royal Sovereign plants were infested with colonies of aphides and mites, which were originally collected from healthy plants, and six clonal virus disease plants were infested with aphides and mites obtained from the same source. As already mentioned, the aphides and mites bred so rapidly that, when the inoculations were due to commence, it was possible to obtain ample mite and aphis material from a single healthy plant and from one virus plant.

DIRECT INOCULATIONS.

The direct inoculations were commenced on June 5th and were continued at weekly intervals concluding on July 21st. Thus seven inoculations were made in all. Five aphides and five mites were transferred to their respective plants on each occasion. Particular care had to be observed when the aphides were transferred, since it will be remembered that mites were also feeding on the same plants from which the aphides were removed, and the possibility of transferring mites along with them had to be avoided. When the transfers were made the aphides and mites were invariably placed on the undersides of the youngest leaflets, near the crowns of the plants. The actual transfers were made by means of a small camel-hair brush under a binocular microscope. Each series of direct inoculations was necessarily a lengthy process since 300 aphides and 300 mites had to be handled each time. To do this properly, that is, without injuring the animals concerned, required a complete day. If weather conditions were not suitable on the day set aside for making the transfers, the work was suspended until more suitable conditions prevailed.

SURVEY OF PLOTS.

The plants were examined each day throughout the growing season in order that any undesirable insect might be detected before it became established on the plants, and also to determine whether any of the aphides were migrating to the mite plots, and to ensure that the control plants were not becoming infested with mites or other insects.

Fortunately, it so happened that all the control plants remained free from attacks of mites and insects throughout the season and, what is more striking, is the fact that the aphis bays did not become infested with mites, nor did the mite bays become infested with aphides. Possibly the arrangement of the trial helped in this direction, it being so arranged that the mite plants were plunged at the east end, with the controls in the centre, and the aphis plants at the west end of the plot.

A small blue weevil (*Rhynchites germanicus* Hb.) was found on a number of plants during the latter part of May, feeding on the petioles of the leaves. Some numbers were removed by hand picking but, as a final precaution, the plants were sprayed with an arsenical wash. The weevil was not noticed again after the first week of June.

In order to prevent stray mites and aphides moving from one bay to another, the soil around and between the rows of bays was moved weekly by means of a hand hoe. In addition, the ground was frequently soaked with a lime-sulphur, nicotine spray.

TREATMENT OF PLANTS.

Since the plants were growing in pots it was necessary to water them twice daily as a matter of routine. All the runners were removed systematically until the end of June. Early in July one sturdy runner was selected from each plant, and was rooted in a three inch pot plunged in the soil next to its parent. All subsequent runners were removed from the plants.

SPREAD OF APHIS AND MITE.

By the end of June the direct effect of the feeding of the aphides and mites became apparent upon the plants. Colonies of aphides and mites were established on each plant in their respective series. Although the degree of infestation was fairly uniform on the aphis and mite bays, it was noticeable that in the series where the mites were taken from healthy plants, the infestation was rather more severe than the series in which the mites were transferred from virus plants. These plants remained more severely infested throughout the season.

The aphis infestation proved to be very similar on both bays, and well established colonies were noted on the petioles and on the undersides of the leaves of each inoculated plant. Two of the plants died in the control series No. I. The complete crowns of these two plants disappeared during one night, and the type of damage suggested that they had been eaten by some animal. Thus the total number of control plants in the two series was reduced to fifty-eight instead of the original sixty.

By the middle of July, the young runners were becoming well established, but this growth was checked to some extent by the aphis and mite infestation. The aphides and mites had made a natural migration from the parent plants via the stolons to the runners. This natural migration obviated the necessity of inoculating the runner plants.

Winged forms of the Strawberry Aphis began to appear towards the end of July, and it was feared that some of them might fly from one aphis bay to the other. To prevent this taking place, some large glass frames were erected between the two aphis bays.

SURVEY OF BAYS AT THE END OF JULY.

A survey of each of the six bays was made at the end of July, and the following details of interest were noted.

Bay 1. Plants inoculated with mites-from-virus plants.

Each of the plants and their runners in this series showed typical direct mite injury. Many of the younger leaflets were so badly injured by the direct feeding of the mites that they turned in colour to a silvery brown and many of them dried out before they had expanded. Many of the older leaves were very much crinkled, were brittle, and were dull in appearance, having lost their natural fresh green colour. The mite population proved to be fairly evenly distributed, but two of the plants were more severely infested than the rest. The runners of these two plants were also very weak.

Bay 2. Plants inoculated with mites-from-healthy plants.

The plants and their runners in this series were almost exactly similar to those described above. The mite infestation was, however, more severe, and in consequence they were somewhat more stunted and crippled. It was quite obvious to the eye that the plants and the runners in both mite series were stunted and in an unhealthy condition. No other explanation could be found for the unhealthy state of the plants ; in all probability, therefore, direct mite injury was the cause.

Bay 3. Absolute " Control " plants (Series I).

All the plants in this series proved to be quite healthy, and the fresh, healthy green of the foliage presented a striking contrast to that on the two mite bays. The runners were also healthy, and were growing vigorously.

Bay 4. Absolute " Control " plants (Series II).

All the plants and their runners in this series proved to be comparable with the controls of Series I, and the vigour and uniformity of these plants was very striking.

Bay 5. Plants inoculated with aphides-from-healthy plants.

The degree of the infestation of aphides on all the plants and their runners in this series was so pronounced that it was amazing to find that the plants were not more severely damaged than they proved to be. Actually, the vigour of these plants was practically as great as that of the control plants. Further, the type of injury caused to the plant by the aphis could readily be distinguished from that caused by the mite. The difference between the two types of damage probably showed up to the best advantage because the two sets of plants were in close proximity to each other, and a quick comparison of the two could be made quite easily. It is doubtful, however, whether the layman could distinguish the two types of symptoms in the field. One of the best symptoms for diagnosing aphis damage is the very peculiar leaf twist which is nearly always produced. This was also present on the young runners. Another method for separating mite and aphis damage is as follows. In the case of a severe mite infestation, multiple crowns are formed, some of which frequently die and, in addition, the plants tend to flatten. The plant does not flatten after a severe aphis attack and multiple crowns are not produced.

Bay 6. Plants inoculated with aphides-from-virus plants.

The degree of aphis infestation proved to be the same on these plants as on those in Bay 5. It was not possible to make an accurate count of the number of aphides present on each plant, since many hundreds were always present.

On July 26th A. Tidy, who had charge of the cultural aspect, reported unprompted that the development of all the plants and runners in Bay 6 was abnormal. A thorough examination of the plants indicated that something peculiar had happened to them, but at the time it was not possible to decide what was the cause of the trouble. The plants were kept under close observation, and four days later it was quite obvious that all the plants and their runners were showing characteristic symptoms of the virus disease. All the plants in this series presented a very marked contrast to those inoculated with aphides-from-healthy plants. The leaves were smaller, and the characteristic "Yellow-edge" symptoms were present, and all the plants were very much "flattened" in appearance. This "flattening" effect was very much more obvious in the runners. Now, if one compared the runners in the aphis-from-healthy plant series with those in the aphis-from-virus plant series, the difference in the height of the plants in the two bays was pronounced. This will be referred to again below.

It will be remembered that the initial inoculations were made on June 5th, and that the virus symptoms were apparent by the end of July, thus the virus must have been transmitted between those two dates, a period of eight weeks. As soon as it was ascertained that all the plants in Bay 6 were infected with virus, another survey of the rest of the bays was made. The two mite bays still exhibited typical mite injury, but there was no indication that any of the plants were infected with virus. There was still the possibility of virus being present, its symptoms being masked by the very pronounced symptoms of direct mite injury; even so, if the virus was present one would have regarded the plants as being suspicious, but observations made in the autumn, a season when the mite symptoms are usually less pronounced and when the virus symptoms are more readily observed, confirmed the opinion that none of these plants showed virus symptoms. Both the control bays still remained in a healthy condition, while the bay inoculated with aphides-from-healthy plants still showed the characteristic leaf twist and curl, which seems to be a peculiar feature of direct aphis injury. Virus symptoms were not noted on any of these plants.

Now, since the whole of the 178 plants used in the investigation were raised from one clone, and since the only plants of this clone which displayed virus symptoms were the thirty which were inoculated with aphides taken from a virus plant, it is clear beyond doubt that the aphides inoculated these plants with virus. All the rest of the plants in this clone were at this time still quite normal.

HEIGHT AND SPREAD MEASUREMENTS.

In order to obtain some permanent record of the difference in vigour of the plants in each series, height and spread measurements were recorded. These records were taken on July 30th and August 30th. These measurements are given in the following Table.

Table showing Height and Spread of Royal Sovereign Strawberry Plants and their Runners.

Inoculated with	Parent Plants.				Runners.			
	Average Date 30–7–34.		Average Date 30–8–34.		Average Date 30–7–34.		Average Date 30–8–34.	
	Height inches.	Spread inches.	Height inches.	Spread inches.	Height inches.	Spread inches.	Height inches.	Spread inches.
Mites from virus plant ..	5·6	14·6	7·1	14·6	3·7	6·9	3·9	6·7
Mites from healthy plant	5·0	14·8	6·6	14·2	3·3	6·7	3·3	8·5
Control (Series I) ..	6·9	15·7	7·3	16·2	4·1	7·9	4·4	10·1
Control (Series II) ..	6·7	16·0	7·0	16·8	4·1	7·5	4·0	9·0
Aphis from healthy plant	6·8	15·3	6·4	16·0	4·1	7·4	4·0	10·1
Aphis from virus plant ..	5·0	15·2	3·8	13·5	2·5	6·2	2·1	7·4

When the records were taken in July, it was quite obvious to the eye that the control plants were larger and more vigorous than the rest of the plants. The visual difference between them and the rest was actually much more striking than the figures in the Table suggest. It will be seen from the Table that the mite-from-healthy plant series and the aphis-from-virus plant series averaged 5 inches in height, while the controls averaged 6·8 inches. The average spread of the plants did not vary to any marked extent, and the small differences were not noticeable in the field.

The second series of records obtained at the end of August showed very marked differences. The average height of the control plants was seven inches, while the mite infested series and the aphis-from-healthy plant series averaged between 6·5 and 7 inches in height. It will be noticed in particular that the aphis-from-healthy plant series were nearly as tall as the control series, which tends to support the suggestion that the aphis alone was not causing much damage to the plants.

The aphis-from-virus plant series presented a very striking contrast to the rest of the plants in the experiment. The average height was under four inches, that is, more than three inches shorter than the control plants. The average spread was also considerably less than that of the controls, but the difference in this respect was not so striking. This can probably be accounted for by the fact that once the plants become infected with virus they become flattened until they lie on the ground and eventually rot away. Again, it will be noted from the spread records in the two mite infested series that these plants were smaller than the controls, as shown by the figures in column five.

PLATE I.

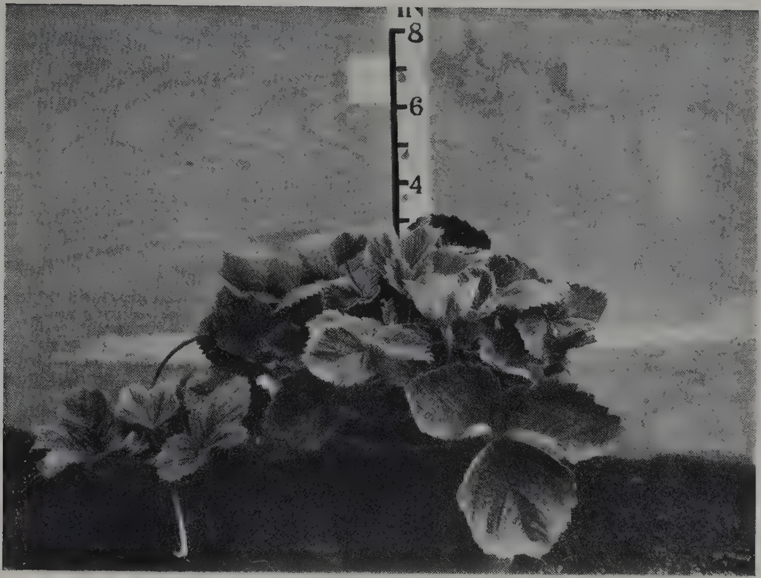


Fig. 1. Inoculated with mites-from-healthy plant.

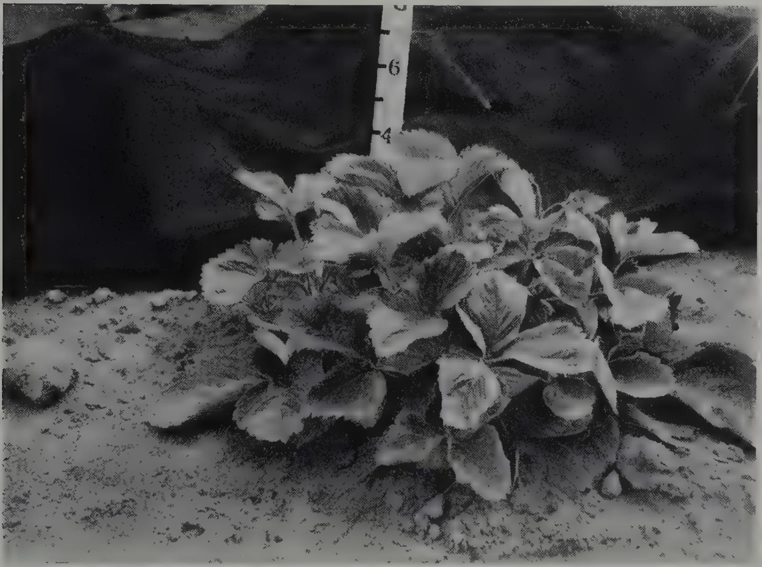


Fig. 2. Inoculated with mites-from-virus plant.

PLATE II.

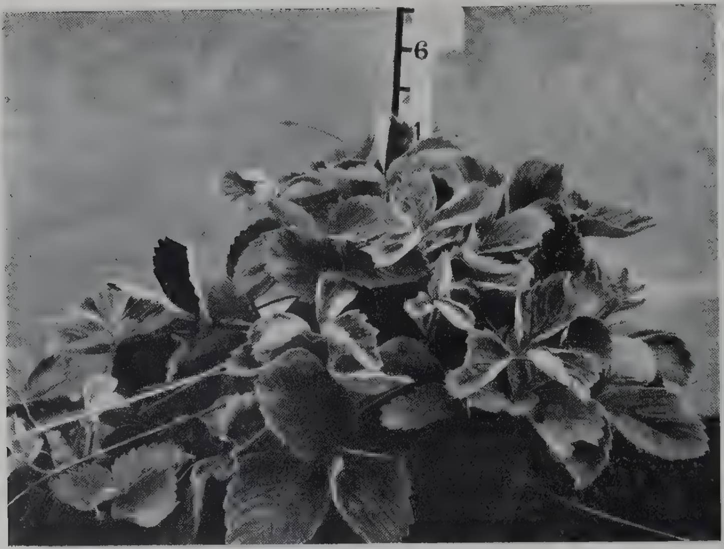


Fig. 1. Inoculated with aphides-from-healthy plant.

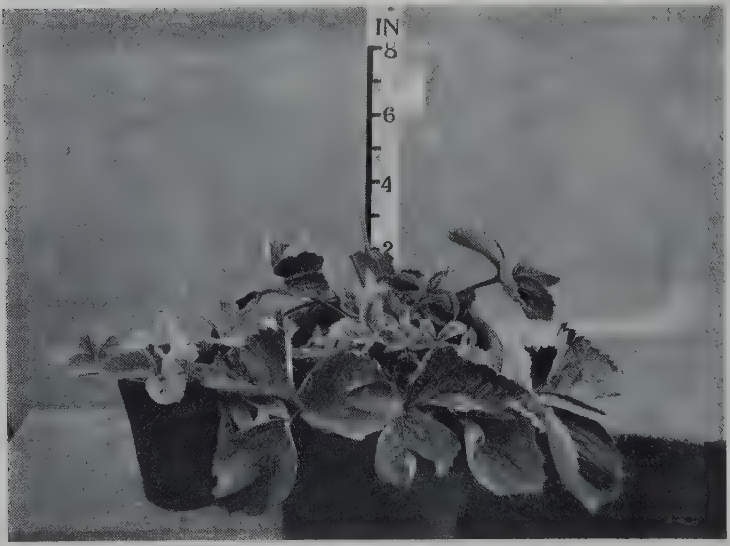


Fig. 2. Inoculated with aphides-from-virus plant.

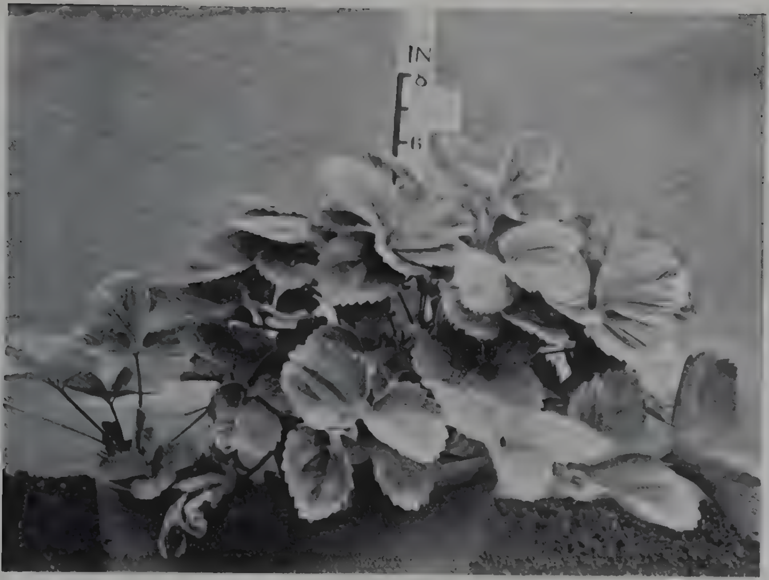


Fig. 1. Untreated "control" plant.

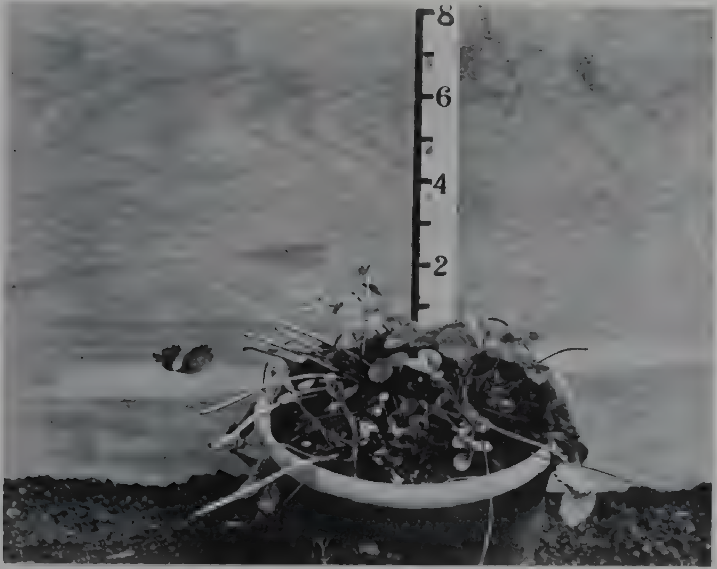


Fig. 2. Source of infection. Virus plant.

The records of the height and spread of the runners are also of interest.

It will be seen that the average height of the aphis-from-virus plant series was 2·5 inches in July, and exactly the same height at the end of August, as compared with an average height of 4 inches in the controls. The constant height figure of 2·5 inches in the virus series can probably be accounted for by the fact that these runners were infected with virus from the very beginning of their existence, and thus they did not have a reasonable chance of growing away. It is also of interest to note that the height and spread of the runners in the aphis-from-healthy plant series were very similar to that of the controls.

FINAL SURVEY OF PLOTS.

Another detailed survey of the plants was made during the first week of September. All the control plants still proved to be quite healthy, and insects or mites were not detected on any of them. A typical specimen control plant is shown in Plate III, Figure 1.

It was noted that the degree of infestation on the two mite bays was becoming much less severe, and some of the plants were actually beginning to make fresh growth. However, the damage caused by the mite earlier in the season was still apparent on the majority of the plants (see Plate I, Figures 1 and 2). None of the plants or their runners in these two series exhibited symptoms of the virus.

In the aphis-from-healthy plant series, one plant only and its runner showed characteristic "Yellow-edge" symptoms. It is difficult to explain how this plant became infected ; it should be noted, however, that this plant happened to be the end one in the row adjacent to the aphis-from-virus plant series, and it is possible, therefore, that a few stray aphides passed from the virus plants round the end of the glass screen, and infected the end one in the next series. (See plan on page 42.)

Plate II, Figure 1, shows a typical plant in the aphis-from-healthy plant series. The peculiar leaf twist effect caused by the aphis stands out clearly in the photograph. In the aphis-from-virus plant series the appearance of the plants was similar to that noted a month earlier. The "flattened" appearance was still very conspicuous, and the yellowing of the foliage was very pronounced. None of the plants showed any sign of recovery, and it was now clearly evident that each of the thirty plants and all the runners were infected with virus. A typical plant from this series is shown in Plate II, Figure 2.

As will be seen then, all these precautions were fully justified. It is clear that this type of experiment can be conducted successfully out of doors, without the use of an insect-proof greenhouse. It is essential, however, that such an experiment should be carried out on a sufficiently large scale.

CONCLUSIONS.

It is suggested that the results of the above experiments prove conclusively that the Strawberry Aphis (*Capitophorus fragariae* Theo.) is a vector of the virus.

The corresponding evidence in respect of the Strawberry Tarsonemid Mite (*Tarsonemus fragariae* Zimm.) was negative.

It remains to explain the generally negative results obtained with the aphides in the 1932-3 experiment. The main point of difference in the inoculation procedure adopted was that in 1933 inoculation was not started till July, whereas in 1934 it was commenced in early June. This suggests that the optimum time for aphis transfer of this virus may be during the month of June. This aspect of the problem will be explored in future experiments. In addition, it proved to be more difficult to get the colonies of aphides established on the plants in 1933, this being partly due to the heavy storms which followed the inoculations on at least two occasions.

DISCUSSION OF RESULTS.

In 1925 Plakidas (5) referred to an obscure disease of the strawberry in California, and he suggested that the Red Spider Mite (*Tetranychus telarius* L.) and the Strawberry Aphis (*Myzus fragaefolii* Ckll.) were strongly suspected as being the carriers of the disease. In a later report published in 1927, Plakidas (6) states that the Strawberry Xanthosis (Yellows) is an insect borne virus disease, the vector in this case being the Strawberry Aphis (*Myzus fragaefolii* Ckll.). In the same article Plakidas states that the red spider is not capable of transmitting Xanthosis.

More recently Vaughan (7) has found that the crinkle disease of strawberry is transmitted by the aphis, *Myzus fragaefolii* Ckll., and Zeller (8) has also demonstrated that another disease of the strawberry, namely Witch's Broom, can be transmitted from one plant to another by the aphis *Myzus fragaefolii* Ckll.

Thus, *Myzus fragaefolii* Ckll. has been shown to be the vector of three virus diseases, namely, "Yellows", "Witch's Broom" and the "Crinkle" disease. While discussing this aphis, it is interesting to note that Hille Ris Lambers (9), an authority on the group Aphididae, considers the English Strawberry Aphis (*Capitophorus fragariae* Theo.) and the American species (*Myzus fragaefolii* Ckll.) to be one and the same species, and that the correct name of the insect should be *Pentatrichopus potentillae* Wlk.

On the other hand, Mr. F. Laing, of the British Museum, is of the opinion that some doubt still exists, and that the type slide of Walker's *Pentatrichopus potentillae* Wlk. is in such a poor condition that an accurate comparison of the two aphides and the type cannot be made. Therefore, it has been decided to retain Theobald's name, and refer to the English species as *Capitophorus* *fragariae* Theo. until the systemalists are agreed as to the correct name of the species.

Another preliminary report entitled Strawberry Dwarf was published by Plakidas in 1928 (10). Strawberry Dwarf was considered by Plakidas to be of a virus nature, and he suggested that a species of aphis may prove to be a vector. The aphis in question is *Aphis forbesi* Weed. In the course of a preliminary experiment, this aphis transmitted the virus, but the number of plants used was not sufficient to form any definite conclusions.

In 1927 Briton-Jones and Staniland (11) referred to the possibility of the English strawberry aphis being a vector of a virus disease.

They mention the recent results of aphis being a carrier of potato virus, and even draw attention in a last minute note to recent work in California which proved that an aphis (*Myzus fragaefolii* Ckll.) was a principal vector. However, they could not reconcile these findings with certain of their own observations where aphis affected plants returned to a healthy condition and produced normal runners.

Such a case is now easily explained if the aphides were migrants from healthy plants, and from these writers' description of aphis damage, as such, it would appear that there was then difficulty in distinguishing between aphis damage as such and virus.

Finally, Harris (2) in proposing the name "Yellow-Edge" for the virus disease described by him emphasizes the close similarity of the symptoms of that disease and those of Xanthosis (Yellows) as described by Plakidas (6), but considered the data then available inadequate to decide finally whether the two diseases were or were not identical.

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SUMMARY.

1. Experiments on the transmission of the strawberry virus "Yellow-Edge" disease by insects and mites are described.

2. The Strawberry Aphis (*Capitophorus fragariae* Theo.) is shown to be a vector of "Yellow-Edge" virus.

3. The aphis is able to transmit the virus in June, but the evidence so far collected tends to suggest that it is not able to transmit the virus during the latter part of July and August.

4. One hundred per cent. of healthy clonal Royal Sovereign plants showed virus symptoms when inoculated with aphides-from-virus plants.

5. Only one plant showed virus symptoms when inoculated with aphides-from-healthy plants.

6. One hundred per cent. of the absolute " control " plants remained healthy throughout the experiment.

7. Direct aphis injury, while showing characteristic symptoms, did not cause any material harm to the plants.

8. The Strawberry Tarsonemid Mite (*Tarsonemus fragariae* Zimm.) did not transmit the virus in the 1933-4 trials ; in each case negative results were obtained.

9. The Strawberry Tarsonemid Mite, however, may cause severe direct injury to the plant, masking any virus symptoms which may be present. For these reasons the mite may be very important.

10. A technique has been worked out whereby certain insect transmission experiments can be conducted in the open, without the aid of an insect-proof greenhouse.

REFERENCES.

(1) *Harris, R. V.* Grafting as a method for Investigating a possible Virus Disease of the Strawberry. Journ. Pom. & Hort. Sci., 1932, 10, 35-41.

(2) ———. The Strawberry " Yellow-Edge " Disease. Journ. Pom. & Hort. Sci., 1933, 11, 56-76.

(3) *Massee, A. M.* Further Observations on the Strawberry Tarsonemid Mite. East Malling Res. Sta. Ann. Rpt. for 1932 (May 1933), 117-31.

(4) ———. The Warm Water Treatment of Strawberry Runners before Planting. East Malling Res. Sta. Misc. Pub., 1934, No. 14, 3.

(5) *Plakidas, A. G.* An Obscure Disease of the Strawberry Plant. Phytopath., 1925, 15, 730.

(6) ———. Strawberry Xanthosis (Yellows), a New Insect Borne Disease. Journ. Agric. Res., 1927, 36, 1057-90.

(7) *Vaughan, E. K.* Transmission of the Crinkle Disease of Strawberry. Phytopath., 1933, 23, 738-40.

(8) *Zeller, S. M.* Preliminary Study on Witch's Broom of Strawberry. Phytopath., 1927, 17, 329-35.

(9) *Hille Ris Lambers, D.* Notes on Theobald's "The Plant Lice or Aphididae of Great Britain." Stylops., 1933, 2, Pt. 8, 169-76, 5 refs.

(10) *Plakidas, A. G.* Strawberry Dwarf. Phytopath., 1928, 13, 439-44.

(11) *Briton-Jones, H. R.* and *Staniland, L. N.* The Effects of the Strawberry Aphis (*Capitophorus fragariae* Theobald) on the Strawberry Plant. Journ. Pom. & Hort. Sci., 1927, 6, 128-36, 2 Pl.

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INVESTIGATIONS ON CHLOROSIS OF FRUIT TREES

V. THE CONTROL OF LIME-INDUCED CHLOROSIS BY INJECTION OF IRON SALTS

By T. WALLACE

In paper No. IV of the present series (1), experiments were described in which it was shown that a considerable degree of control over lime-induced chlorosis could be obtained by the use of cover crops and permanent grass.

There are many cases, however, in which it is not possible or is undesirable to employ such cultural methods as, for example, in gardens where the ground is used for intercrops and for such fruits as plums where a high level of cultivation is desired.

In view of these facts, other methods of control have been sought which may be applied in such cases and of these special attention has been given to a method described by J. P. Bennett, which appeared to be of promise (2).

This method consists of the injection of various amounts of powdered citrate of iron, either ferrous or ferric citrate, into the wood of the trunk or branches of affected trees, the dose being adjusted according to the diameter of the trunk or branch to be treated.

The method is extremely quick and simple to carry out. It only entails the boring of one or more holes in the trunk or branch to be treated with a brace and bit, introducing the prescribed amount of powdered citrate and plugging the holes with grafting wax. The treatment is best carried out during the dormant season, since the risk of damage from it is then at a minimum.

The doses, number of holes, etc., recommended by Bennett are given in Table I, which is extracted from his original paper.

Bennett points out in his paper that the dosage and technique adopted have been developed mainly from experience with pear trees and adjustments may be required when dealing with other kinds. He states that "about 75,000 pear trees have been successfully treated by the method, mostly by growers themselves".

The improvement effected by the treatment is not to be regarded as permanent but usually lasts for about three years when the process may require repeating.

Table I.

Dosage and Holes for Treating Non-Bearing Chlorotic Trees by the Dry-Salt Method* (after Bennett).

Tree diameter, inches.	Holes.			Amount of Salt.	
	Number.	Diameter, inches.	Depth, inches.	Per hole, ounces.	Per tree, ounces.
1	1	$\frac{1}{4}$	$\frac{5}{8}$	0·01	0·01
$1\frac{1}{2}$	1	$\frac{1}{4}$	1	0·02	0·02
2	1	$\frac{7}{16}$	$1\frac{1}{4}$	0·05	0·05
3	3	$\frac{7}{16}$	$1\frac{1}{4}$	0·03 +	0·10
4	4	$\frac{7}{16}$	$1\frac{1}{2}$	0·05	0·20
5	5	$\frac{7}{16}$	$1\frac{3}{4}$	0·06	0·30
6	6	$\frac{7}{16}$	2	0·08 +	0·50
7	7	$\frac{7}{16}$	2	0·08 +	0·60
8	8	$\frac{7}{16}$	$2\frac{1}{2}$	0·09	0·70
9	9	$\frac{7}{16}$	$2\frac{1}{2}$	0·09	0·80
10	10	$\frac{7}{16}$	$2\frac{1}{2}$	0·10	1·00
15	15	$\frac{7}{16}$	3	0·10	1·50
20	20	$\frac{7}{16}$	3	0·10	2·00

* The dosage is reduced to half or two-thirds of that recommended in the table for Bearing Trees if injury to blossom is feared.

The experiments described in the present paper were carried out at four centres on apples, plums and pears, the treated trees in each case being severely affected by chlorosis and in very poor condition. The injections were made in March 1933, immediately prior to buds bursting, and records on the condition of the trees are available for the seasons, 1932, 1933 and 1934.

The results obtained are similar to those described by Bennett and indicate that the method may be of considerable commercial value.

EXPERIMENTAL.

The Centres.

Centre A. Two experiments were carried out at this centre:

(a) On a block of 40 apple trees, variety chiefly James Grieve with a few Worcester Pearmain. The trees were in two rows of 20, planted at 15 ft. square, about 10 years old, and had been trained originally as espaliers. Practically every tree was more or less chlorotic, often severely, and showing die-back.

For the purpose of the experiment an area containing twelve trees in each row was allowed to become overgrown with grass and weeds and the remainder kept under cultivation. The trees in the cultivated group, with the exception of Nos. 13 and 33 which were treated as buffers between the two plots, were given the injection treatment. The object of the weed treatment was to compare the effects of this with the injection treatment.

(b) Eight pear trees, variety Conference, about 5 years old, trained as cordons and growing in one row of a block, planted 30 in. apart in the row and rows 5 ft. apart, were treated. They were all very poor trees, and severely chlorotic.

The area at Centre A is part of a commercial fruit plantation. The site is a gentle to medium slope to the south. The soil is mainly derived from Keuper Marl with a slight admixture of Bunter sand and gravel. The surface soil contains about 1 per cent. $CaCO_3$, but there are bands of soft calcareous rock in the subsoil layers.

Centre B. Seventeen plum trees, growing in a commercial plantation, were treated. Fifteen were of the variety Pershore Egg and two of the variety Victoria. The trees are very old, probably over 50 years, and were generally large specimens but showed much chlorosis, dying back of large branches and the wood of both trunks and branches was often rotten and many trunks were hollow.

The site is slightly elevated and level, the soil being derived from a local Boulder Clay which is mainly a mixture of Keuper Marl and Lower Lias Clay and is highly calcareous.

Centre C. The plot is located in a garden and contains nine bush apple trees, varieties Newton Wonder and Gascoigne's Scarlet, between 20 and 30 years of age. The trees are planted in 3 rows each containing 3 trees, and had previously made fairly strong growth, although for many years showing a fair amount of chlorosis and this was generally severe just prior to treatment, shoot growth being greatly restricted.

The site is rather low lying and level, and the soil derived from a highly calcareous Lower Lias Clay. The plot is highly cultivated for the production of bulbs.

Centre D. The trees at this centre are all bush apple trees and occur in three blocks of different ages in a garden.

(a) *Block I.* The block contains nine trees which have been under observation for several years. They are about 20 years old and, previous to 1928, were growing under cultivated conditions, when chlorosis was very severe. Since then they have been under grass, treated like a lawn, and most of the trees have done well, but two have continued to grow very badly although only one of these has shown appreciable chlorosis. These two trees were selected for treatment.

(b) *Block II.* These trees are about 10 years old and for the greater part of the time have been grown under conditions of a grass lawn. Two trees of the eight which have not grown well and show chlorosis were treated.

(c) *Block III.* These trees form a block of miscellaneous varieties, about 5 years old from planting and growing under conditions as in Block II. Six trees showing varying degrees of chlorosis and poor growth were selected.

The site of this centre is somewhat elevated, but practically level, the soil being derived from Dolomitic Conglomerate, very shallow and containing a high proportion of carbonates.

METHOD OF TREATMENT.

The methods used in making the injections were similar to those described by Bennett, slight modifications being made to suit the circumstances. Ferric citrate was used. This was purchased as " scales " and these finely ground to a powder in a laboratory mortar. For convenience in measuring the doses in the field, a series of holes of appropriate sizes were made in the ends of corks so as to hold the amounts likely to be used when the holes were filled with powder flush to the surfaces with gentle tapping.

The tree holes were bored in trunks and branches by means of a brace and bit, bits ½ in. and ⅜ in. respectively being used for larger and smaller limbs. Where more than one hole was bored in any trunk or branch these were made at slightly different levels so that if damage occurred to the wood the treated limb would not be ringed. The bore holes always pointed slightly downwards so that the powder could be easily placed in the bottoms.

To introduce the powder into the holes, it was poured on to a piece of smooth paper and this tilted into a glass adaptor tube with slightly narrowed end which was fitted into the hole so as to touch the bottom. The powder was gently tapped down the tube, the last portions being pushed out into the hole with a camel-hair brush and a piece of copper wire. The powder was finally pressed with a flat-ended glass rod and a small cork inserted in the hole.

As the treatments were carried out rather late in the dormant season, from March 13th-22nd, and the buds were practically at the bursting stage and some even burst, the amounts of powder given were generally only about half to three-quarters of those recommended by Bennett and in certain cases no doubt could be increased with advantage.

The operations were found easy to perform and, given dry still weather, could be carried out easily by unskilled labour.

The details of the trees, number of holes, doses per hole and a comparison of the total doses per tree with those recommended by Bennett are shown in Table II, a, b, c, d.

TABLE II.

Showing Details of Trees and Injection Treatments.

a. *Centre A.*

Tree No.	Particulars of Tree.	Diameter. Trunk.	Holes. No. and Depth.	Dose per Hole. oz.	Total Dose. oz.	Total Dose recommended by Bennett. oz.
APPLES.						
Row 1, T. 14	All trees fairly small,	3″	2 ; 1¼″	0·03	0·06	0·10
15	bearing branches with	2½″	2 ; 1¼″	0·03	0·06	0·05–0·1
16	restricted shoot growth	3″	2 ; 1¼″	0·03	0·06	0·1
17	and some die-back.	3″	2 ; 1¼″	0·03	0·06	0·1
18	Many trees branches	3½″	3 ; 1¼″	0·03	0·09	0·1–0·2
19	removed for canker	3″	2 ; 1¼″	0·03	0·06	0·1
20		3½″	3 ; 1¼″	0·03	0·09	0·1–0·2
Row 2, T. 34		4″	2 ; 1½″	0·05	0·10	0·2
35		3½″	3 ; 1¼″	0·03	0·09	0·1–0·2
36		4″	3 ; 1½″	0·05	0·15	0·2
37		3½″	3 ; 1¼″	0·03	0·09	0·1–0·2
38		3½″	3 ; 1¼″	0·03	0·09	0·1–0·2
39		4″	3 ; 1½″	0·05	0·15	0·2
40		3¼″	2 ; 1¼″	0·03	0·06	0·1 +
PEARS.						
Row 3, T. 24	All trees bear stunted	1½″	1 ; 1″	0·01	0·01	0·02
26	shoots and some die-	2″	1 ; 1¼″	0·03	0·03	0·06
31	back. Average height	1¼″	1 ; ¾″	0·01	0·01	0·01–0·02
32	6 feet	1½″	1 ; 1″	0·01	0·01	0·02
33		1½″	1 ; 1″	0·01	0·01	0·02
34		2″	1 ; 1¼″	0·03	0·03	0·06
35		1½″	1 ; 1″	0·01	0·01	0·02
36		1¼″	1 ; ¾″	0·01	0·01	0·01–0·02

b. *Centre B.*

Tree No.	Particulars of Tree.	Diameter. Trunk.	Holes. No. and Depth.	Dose per Hole. oz.	Total Dose. oz.	Total Dose recommended by Bennett. oz.
Row 2, T. 18	Large, full head. Centre of trunk hollow	10″	5; 2½″	0·10	0·50	1·0
9	Previously large tree but all branches removed and now only small shoots. Centre of trunk rotten	9″	5; 2½″	0·09	0·45	0·80
8	Smaller tree than 9. All branches removed and only small shoots. Loose in ground. Centre of trunk rotten	6″	3; 2″	0·08	0·24	0·50
17	Large, full head. Wood stained brown	7″	4; 2″ 2; 1½″ (branch)	0·07 0·05	0·38	0·60
19	Previously large; 3 large branches remain. Wood partly soft and partly firm but brown	8″	4; 2½″	0·09	0·36	0·70
Row 4, T. 2	Large tree; 3 branches. Not possible take trunk diameter	5″ 5″ 4″ (branches)	3; 1¾″ 3; 1¾″ 2; 1½″	0·05 0·05 0·05	0·15 0·15 0·10	0·30 0·30 0·20
5	Previously large. All branches removed, only small shoots. Wood partly brown	8″	4; 2½″	0·09	0·36	0·70
7	Previously large tree. Now topped. 3 main branches. Wood mostly rotten. Only branch diameters taken	5″ 4″ 4″ (branches)	3; 1¾″ 2; 1½″ 2; 1½″	0·05 0·05 0·05	0·15 0·10 0·10	0·30 0·20 0·20
8	Large tree; practically full head	8″	4; 2½″	0·09	0·36	0·70
11	Large tree, partly topped, 2 main branches. Only branch diameters taken	5″ 3½″ (branches)	3; 1¾″ 2; 1¼″	0·05 0·03	0·15 0·06	0·30 0·15
13	Originally two main branches, one remains and this partly cut back. Branch diameter only taken	5″ (branch)	3; 1¾″	0·05	0·15	0·30
22	Large tree; no main trunk, 2 main branches	6″ 4″ (branches)	3; 2″ 2; 1½″	0·08 0·05	0·24 0·10	0·50 0·20
23	Large tree; no main trunk, 2 main branches. Wood mostly rotten	6″ 5″ (branches)	3; 2″ 3; 1¾″	0·08 0·05	0·24 0·15	0·50 0·30
24	Previously fairly large tree. Practically half of top cut away and growth poor	7″	4; 2″	0·07	0·28	0·60
15	Variety Victoria. Small tree, poor top	5″	3; 1¾″	0·05	0·15	0·30
17	Variety Victoria. Fairly good top	5″	3; 1¾″	0·05	0·15	0·30
19	Very large tree. Full head. No trunk, 2 main branches. Wood slightly rotten, smaller branch	9″ 5″ (branches)	5; 2½″ 3; 1¾″	0·08 0·05	0·40 0·15	0·80 0·30

c. *Centre C.*

Tree No.	Particulars of Tree.	Diameter. Trunk.	Holes. No. and Depth.	Dose per Hole. oz.	Total Dose. oz.	Total Dose recommended by Bennett. oz.
No. 1	Newton, Large tree	8″	8; 2½″	0·09	0·72	0·70
2	Newton, large tree. No trunk measurement possible	5½″ 5″ 5″ (branches)	3; 1¾″ 3; 1¾″ 3; 1¾″	0·06 0·05 0·05	0·18 0·15 0·15	0·3–0·5 0·30 0·30
3	Newton, large tree	8″	4; 2½″	0·09	0·36	0·70
4	Gascoigne, large tree. Some canker	8″	4; 2½″	0·09	0·36	0·70
5	Ditto	6″	6; 2″	0·08	0·48	0·50
6	Ditto	7″	4; 2″	0·07	0·28	0·60
7	Newton, large tree	8½″	5; 2½″	0·08	0·40	0·7–0·8
8	Ditto. No trunk measurement possible	8½″	4; 2½″	0·09 (3 holes) 0·07 (1 hole)	0·34	0·7–0·8
		7″ (branches)	4; 2″	0·07	0·28	0·60
9	Newton, large tree	8″	4; 2″	0·09	0·36	0·70

d. *Centre D.*

Tree No.	Particulars of Tree.	Diameter. Trunk.	Holes. No. and Depth.	Dose per Hole. oz.	Total Dose. oz.	Total Dose recommended by Bennett. oz.
Block I.						
No. 4	Wellington. Poor condition	4″	4; 1½″	0·05	0·20	0·20
7	King Pippins. Poor condition, some die-back	5½″	5; 1¾″	0·06	0·30	0·3–0·5
Block II.						
7	Variety, James Grieve. Poor condition. Some die-back	2″	1; 1¼″	0·04	0·04	0·05
8	Variety, Rich's Favourite. Restricted shoot growth	2½″	2; 1¼″	0·03	0·06	0·05–0·10
Block III.						
1	Variety, Ashmead Kernel. Medium growth	2½″	2; 1¼″	0·03	0·06	0·05–0·10
2	Variety, Mr. Gladstone. Medium growth	3″	2; 1¼″	0·03	0·06	0·10
5	Variety, Feltham Beauty. Shoots restricted	2″	1; 1¼″	0·03	0·03	0·05
6	Variety, Allington Pippin. No shoot growth	1¾″	1; 1″	0·02	0·02	0·02–0·05
11	Variety, Ellison's Orange. Shoots restricted	2¾″	2; 1¼″	0·03	0·06	0·10—

RESULTS.

The records obtained for the individual trees at the four centres, relating to shoot growth and chlorosis for the seasons 1932, 1933 and 1934, and to cropping for 1934, are presented in Table III, a, b, c, d. Since the injection treatments were given in March 1933, the growth and chlorosis records refer to the season immediately prior to the treatment and to two others immediately following. The crop records for 1934 are inserted since the crops in that season were medium to heavy at all centres and these might have been expected to place a heavy strain upon trees not in good condition.

TABLE III.

Showing Records for Shoot Growth, Chlorosis and Cropping, Seasons 1932-4.

a. *Centre A.*

	2.9 32		8.9.33		27.7.34		
Tree No.	Shoot Growth.	Chlorosis.	Shoot Growth.	Chlorosis.	Shoot Growth.	Chlorosis.	Crop.
APPLES.							
Cultivated Area.							
Row 1, T. 13	M.	M.	F.G.	S.—M.	F.G.	M.	L.
14*	M.	B.	V.G.	T. one shoot	V.G.	T.—V.S. many shoots	L.
15*	M.	B.	V.G.	V.S. south side	V.G.	S.—M.	V.L.
16*	B.	B.	V.G.	T. small shoots	V.G.	T.—V.S. young shoots	Nil
17*	B.	B.	V.G.	V.S. south branch	V.G.	S. few shoots S.—M.	L.
18*	F.G.	M.	V.G.	T. centre branch	V.G.	T.—V.S. few shoots.	V.L.
19*	F.G.	M.	G.	T. weak shoot south side	V.G.	T. one young shoot	L.
20*	G.	S.	V.G.	T. north side	V.G.	Green	
Row 2, T. 33	G.	S.—M.	F.G.	M.—B.	F.G.	M. to M.—B.	M.
34*	F.G.	B.	V.G.	T. south side	F.G.	T.—V.S. south side	M.
35*	F.G.	M.	V.G.	T. lower shoots	V.G.	S.—M. north side	
36*	M.	B.	V.G.	T. two branches	V.G.	T. south side	M.
37*	M.	M.—B.	V.G.	T. south side	G.	S.—M. young shoots	M.
38*	G.	S.	V.G.	Green	V.G.	Green	M.
39*	G.	M.—B.	V.G.	Green	V.G.	Green	M.
40*	G.	T.	V.G.	Green	V.G.	Green	H.

* Denotes injected apple trees.

a. *Centre A* (continued).

	2 9 32		8 9 33		27 7 34		
Tree No.	Shoot Growth.	Chlorosis.	Shoot Growth.	Chlorosis.	Shoot Growth.	Chlorosis.	Crop.
Grassed Area.							
Row 1, T. 1	B.	Green	F.G.	Green	F.G.	Green	L.
2	F.G.	Green	F.G.	Green	V.G.	Green	M.
3	F.G.	T.	G.	Green	G.	Green	M.
4	B.	S.	F.G.	Green	F.G.	Green	Nil
5	F.G.	S.	G.	Green	V.G.	Green	H.
6	B.	B.	F.G.	T.	F.G.	T. young shoots	H.
7	F.G.	S.	G.	V.S. south side	G. except south side	M. south side	M.
8	B.	M.	F.G.	Green	V.G.	T. north half	M.
9†	B.	B.	F.G.	Trace	F.G.	S. especially south side	M.
10	M.	M.	M.	V.S.	F.G.	V.S. young shoots	M.
11	M.	B.	M.	M.	F.G. (canker)	V.S. south side	M.
12	B.	B.	M.	V.S.	F.G.	T.—V.S. young shoots	V.L.
21	B.	Green	M.—B.	Green	M.—B.	Green	Nil
22	F.G.	S.	G.	Green	V.G.	Green	M.
23	F.G.	S.	G.	T. at tips	V.G.	T.	M.
24	M.	S.—M.	M.	Green	V.G.	Green	H.
25	M.	B.	M.	S.	V.G.	Green	M.—H.
26	B.	B.	F.G.	T.	V.G.	V.S. some young shoots	H.
27	M.	B.	M.—B.	M.	G. except centre	S.—M.; M. poor branches	M.
28	B.	B.	Part F.G. Part B.	S. on poor branches	V.G. except one branch	S. poor branches	M.
29†	M.	B.	M.	M.	F.G.—G.	M.	L.
30	M.	B.	F.G.	T.	G.	T.	M.
31	F.G.	S.	G.	Green	G.	Green	L.—M.
32	B.	B.	Part F.G. Part B.	M.—B.	G.	M.	L.

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† 1933/4 grass cover for trees Nos. 9-12 and 29-32 very poor. In 1934 grass practically finished at Trees 11 and 31.

a. *Centre A* (continued).

Tree No.	2.9.32		8.9.33		27 7 34		
	Shoot Growth.	Chlorosis.	Shoot Growth.	Chlorosis.	Shoot Growth.	Chlorosis.	Crop.
PEARS.							
Row 3, T. 24	B. Die-back	B.	V.G.	T.	V.G.	T.	M.
26	M.	B. branch s.w. side	V.G.	T.	V.G.	Green	M.
31	M.	V.B.	F.G.	Green	F.G.	Green	H.
32	B.	B.	F.G.	T.	G.	Green	M.
33	B.	B.	F.G.	T.	F.G.	Green	M.
34	M. Some die-back	B.	Part V.G. Part M.	V.S. on weak side of tree	V.G.	T.	H.
35	B.	B.	F.G.	Green	F.G.	T.	M.
36	B. Die-back	V.B.	M.	Green	V.G.	Green	Nil

b. *Centre B.*

Tree No.	24.8.32	8.9.33		27 7 34		
	Chlorosis.*	Shoot Growth.	Chlorosis.	Shoot Growth.	Chlorosis.	Crop.
Row 2, T. 8	B.	G.	V. slight	V.G.	T. one branch	H.
9	B.	G.	T.	V.G.	Green	H.
17	M.	G.	T.	V.G.	T. top few shoots	H.
18	B.	G.	Slight one branch	V.G.	T. one branch	H.
19	M.	V.G.	T. on suckers	V.G.	Green	H.
Row 4, T. 2	M.	V.G.	T. on suckers	V.G.	T.	V.H.
5	B.	F.G.	Slight on weak branch	V.G.	T.	H.
7	M.	F.G.	T. one shoot	G.	Green	H.
8	M.	G.	T. on weak shoot	G.	T. one branch	H.
11	M.—B.	G.	Green	V.G.	Green	H.
13	B.	B.	T. few shoots	M.	T. few leaves	H.
22	M.	F.G.	T. on suckers	G.	T. on suckers	H.
23	M.	G.	T. on suckers	G.	T. on suckers	H.
24	B.	M.	T. on few small shoots	M.	T. on suckers	V.H.
Row 5, T. 15	M.	M.	Green	F.G.	T.	H.
17	M.	F.G.	T. tip one shoot	F.G.	T. tips lower branches	H.
Row 6, T. 19	M.	G.	T. one shoot	V.G.	T. one shoot	H.

* All trees used for experiment in very poor condition. Those marked Chlorosis B. generally appeared to be dying and showed much die-back of large branches.

c. *Centre C.*

	2.9.32	8.9.33		27.7.34		
Tree No.	Chlorosis.	Shoot Growth.	Chlorosis.	Shoot Growth.	Chlorosis.	Crop.
No. 1	B.	V.G.	T.	V.G.	V.S.	M.
2	B.	V.G.	T.	V.G.	S.	M.
3	B. one side only	V.G.	T.	V.G.	S.—M. one side only	M.
4	B.	V.G.	S.	V.G.	S.—M. part of tree	V.L.
5	B.	V.G.	T.	V.G.	V.S.	M.
6	B.	V.G.	S.	V.G.	V.S.	L.
7	M.	V.G.	T.	V.G.	V.S.	L.
8	S. one side	V.G.	T.	V.G.	Green	H.
9	T.	V.G.	T.	V.G.	Green	H.

d. *Centre D.*

	10.7.32		27.9 33		4 8 34		
Tree No.	Shoot Growth.	Chlorosis.	Shoot Growth.	Chlorosis.	Shoot Growth.	Chlorosis.	Crop.
Block 1.							
No. 4	B.	T.	Dead	—	—	—	—
7	B. Die-back	M.—B.	G. except one branch	Green	V.G.	Green	H.
Block 2.							
No. 7	B. Die-back	B.	B.	Green	G.	Green	M.
8	B.	M.	V.G.	Green	V.G.	Green	M.
Block 3.							
No. 1	M.	S.	V.G.	Green	V.G.	Green	Nil
2	M.	S.	V.G.	Green	V.G.	Green	Nil
5	M.	M.	V.G.	Green	V.G.	Green	M.—H.
6	B.	M.	G.	Green	V.G.	Green	L.
11	M.	S. to S.—M.	V.G.	Green	V.G.	Green	Nil

Shoot Growth.—V.G. = Very Good ; G. = Good ; F.G. = Fairly Good ; M = Medium ; B. = Bad.

Chlorosis.—Green = No Chlorosis ; T. = Trace ; V.S. = Very Slight ; S. = Slight ; M. = Medium ; B. = Bad.

Crop.—H. = Heavy ; M = Medium ; L = Light.

In Table IV an attempt has been made to summarize the results for shoot growth and chlorosis by substituting numerical values for those given in the detailed tables. By this method an average value per tree can be calculated for each season and these show in a clear way the seasonal trends for growth and chlorosis.

The numerical values given are as follows:

Shoot growth: Very good = 4; Good = 3; Fairly Good = 2½; Medium = 2; Bad = 1.

Chlorosis: Green = 0; Trace = ½; Very Slight = 1; Slight = 2; Medium = 3; Bad = 4.

TABLE IV.

Summary of Records for Shoot Growth and Chlorosis.
Average Markings per Tree.

Plot.	Shoot Growth.			Chlorosis.		
	1932	1933	1934	1932	1933	1934
Centre A.						
Plot a.—Apples.						
Grass Plot—24 trees	1¾	2¼	3¼	2¾	1	1
Cultivated Plot. Injected trees—14 trees	2¼	4	3¾	3¼	½	1
Plot b.—Pears—8 trees	1½	3	3¼	4	½	¼
Centre B—Plums.						
17 trees	—	2¾	3¼	3½	¾	½
Centre C—Apples.						
9 trees	—	4	4	3¼	¾	1¼
Centre D—Apples.						
Block 1—2 trees*	1	3	3	3½	0	0
Block 2—2 trees	1	2½	3½	3½	0	0
Block 3—5 trees	2	4	4	2½	0	0

* One tree died, thus records only for one tree.

DISCUSSION.

The results as summarized in the tables show that the injection treatment has greatly improved the trees in all the experiments and in fact in all cases it has converted typical failures due to chlorosis into normal, healthy specimens. The main point of interest now lies in the time over which the treatments will remain effective, there being indications in two cases, Centre A, Plot *a*—apples, and Centre C—apples, that the chlorotic conditions are returning. In this connection it should be noted that the doses applied have only been from one half to slightly over this amount of those recommended by Bennett, and since it appears that the doses could be safely increased, in all probability more lasting effects can be obtained.

A further point has emerged from the observations which is that special attention must be paid to the positions at which the injections are made. In these experiments, in most of the cases where chlorosis was recorded as "Trace" or "Slight" after injection treatment, the symptoms were confined to branches which had obviously been "missed" by the treatment. Improvement in this respect would undoubtedly be achieved by placing the holes in positions for particular purposes as suggested by Roach (3). This point is being tested in further experiments.

The main points in connection with the individual centres are as follows:

Centre A. Plot a. Apples. Both the grass and injection treatments were effective in the season of the treatment, 1933, the results from the latter being especially excellent. In 1934, there was some return of the chlorosis on the injected trees but those under grass remained as in 1933. In the grassed area it should be noted that the grass cover has been very poor in both seasons, especially for trees 9-12 and 29-32. Shoot growth continued to improve on the grassed trees in 1934.

Centre A. Plot b. Pears. The results here have been excellent but in 1934 cultivation was neglected and the plot was very weedy, which condition may have assisted in checking chlorosis.

Centre B. In spite of the fact that these trees are very old and much of the heart wood rotten, the effects produced have been very marked and the trees appear to have become rejuvenated. The only chlorosis present is on "missed" branches and in 1934 both shoot growth and crop were excellent.

Centre C. The effects observed in 1933 were excellent, the treatment resulting in very vigorous shoot growth, which was maintained in 1934, and the disappearance of chlorosis excepting from shoots which were probably "missed".

There was a very definite return of the chlorotic conditions in 1934 on young growths and maybe increased doses will be required at this centre.

Centre D. Apart from the death of one tree in 1933, immediately following the treatment, the results have been excellent. Chlorosis, so far, has been entirely cured and the trees are healthy and vigorous. The killed tree, it should be noted, has been in a very weak condition for many years, and in any case was quite useless. It should be observed that at this centre all the trees are already under "grass lawn" conditions, which had already greatly improved the conditions of most of the trees on the plot, but had failed to produce satisfactory results in the treated specimens. It is suggested that a combination of injection and covercrop treatments may provide an excellent solution of the trouble in many cases where covercrops are possible.

The results obtained in these experiments indicate that the method of dry salt injection is of considerable value as a means of controlling lime-induced chlorosis and should be useful in commercial plantations.

ACKNOWLEDGMENTS.

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REFERENCES.

(1) *Wallace, T.* Investigations on Chlorosis of Fruit Trees. IV. The Control of Lime-induced Chlorosis in the Field. Journ. Pom. & Hort. Sci., Vol. VII, No. 4 (1929).

(2) *Bennett, J. P.* The Treatment of Lime-induced Chlorosis with Iron Salts. Univ. of California Agric. Expt. Sta. Circular 321 (March 1931).

(3) *Roach, W. A.* Injection for the Diagnosis and Cure of Physiological Disorders of Fruit Trees. Ann. App. Biol., Vol. 21, p. 333 (1934).

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FURTHER STUDIES OF THE BROWN-ROT FUNGI

VII. A SHOOT WILT IN STOOLS AND LAYER BEDS OF PLUM STOCKS, AND ITS RELATION TO WITHER TIP

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INTRODUCTION.

THE disease described in the present paper was observed as long ago as 1924. The fungus associated with the disease was isolated from affected shoots the same year and various inoculation experiments were carried out but without any conclusive results, the material available proving unsuitable for critical experiments. It was decided therefore to raise plants that would be comparable with those in the field and yet could be easily manipulated and kept under controlled conditions. This took considerable time and it was not until 1933 that results were obtained resembling those observed in the nursery rows and sufficiently clear-cut to warrant the conclusion that the organism isolated was the cause of the disease.

In raising plum stocks vegetatively, by means of stools or layers, the older parts of the plants are below soil level and only the current year's shoots grow out into the air. During the growing season these shoots are " earthed up " from time to time, in order to induce the development of roots from their lower parts. The operation as practised at that time and still in use with slight modification is as follows. The layered stems (pegged down) and the crowns of stools are left exposed during winter until about the end of February, when they are covered with an inch or so of soil, the buds being still dormant ; the shoots grow through this layer and when they are from four to six inches high (towards the end of May) two or three inches of soil are earthed round them, and more soil is applied about a month later. At the end of the season these rooted one year old shoots are all (except those retained for continuing the layering) removed from the parent stools or layers and planted out in nursery rows to serve as stocks, which are later budded with commercial varieties as scions.

Round about 1924 many varieties of plums were being propagated in this way at the East Malling Research Station in order to test their suitability as stocks for the various varieties of plums and peaches in common cultivation. The Shoot Wilt observed on these stocks was recorded for 1924 (4), and a brief account of it has more recently appeared (5), but hitherto no description of the experimental evidence as to the cause of the disease has been published.

THE DISEASE IN THE NURSERY.

About the middle of June 1924 many shoots in these stool beds and layer rows were dead or dying. On the dead shoots the leaves were brown, curled and withered ; on others the leaves were green and just beginning to flag. The diseased shoots were here and there in the rows, usually only one to a stool or layer, but occasionally two, rarely three, were found in close proximity. Most stools and layers were quite free from the disease and on none were all the shoots killed. There was no evidence therefore that the disease started in definite spots and then spread outwards from those spots as centres ; it appeared rather that the infection was localized to the individual wilted shoots.

On a healthy stool shoot that part below soil level (except perhaps that portion most recently covered with earth) is more or less etiolated, pale (whitish), and leafless, the leaves formerly present having rotted away in the soil.

Wilted shoots were pulled up for examination and on the normally etiolated lower part of each was found a discoloured area several centimetres long and completely encircling the shoot. Such a lesion was sufficient to account for the wilting of those parts above it.

VARIETIES AFFECTED.

All the varieties of rootstocks that were being raised at that time were affected to some extent. These varieties included Broad Leaved Mussel, Common Mussel, Narrow Leaved Mussel, Common Plum, St. Julien de Toulouse, Warwickshire Drooper, Cambridge Gage, Pershore, Brussels, Brompton, Myrobolan, Black Damas and Cluster Damson. Few diseased shoots were found on the Myrobolan selections, Myrobolan A being free from the disease. The variety most severely infected was Black Damas D.

ISOLATION OF THE FUNGUS.

The first shoot examined critically showed a blackened area extending from ground level downward for 5 cm. On making sections through this portion, fungal hyphae were found in the tissues, so attempts were made to isolate the fungus. The affected part of the shoot was cleansed on the surface by sponging with cotton wool moistened with 95 per cent. alcohol. Sections were cut and particles of these were teased out in sterilized water, then placed on prune agar plates. Two of these gave rise to fungal growth with some bacterial colonies. As the fungus grew away from the bacteria it produced the lobing and zonation that are characteristic of plate cultures of *Sclerotinia laxa* Ader. and Ruhl. [=*Sclerotinia cinerea* (Bon.) Schröt.].* For confirmation as to the identity of the fungus, transfers were made to sterilized potato plugs and after a few days the resulting cultures produced the grey tufts of monilioid chains of conidia typical of the Monilia stage of *S. laxa*.

Later the same fungus was isolated from a number of these underground lesions and it seemed probable therefore that *S. laxa* was the cause of the wilting.

That a Brown Rot fungus, commonly associated with fruit rot and blossom wilt, should be found living underground was in itself of theoretical interest, and since the infection resulted in the death of nursery stocks, the disease was of practical importance and warranted further investigation.

Two possible explanations of the manner in which infection occurred presented themselves. Either *S. laxa* infected directly from the soil which would imply that it may sometimes exist as a soil fungus ; or it infected the lower leaves shortly before they were covered with soil, and, extending into the underground parts of the shoots formed the lesions which caused the collapse of those parts above. The latter explanation seemed the more plausible, and experiments were based on that assumption.

Wither Tip in Relation to the Wilting of Stool Shoots.

That *Sclerotinia laxa* is able to infect plum leaves and extend into the shoots bearing them has been known for some time and the diseases recognized as Wither Tip (2) and Spur Blight or Shoot Wilt (3) are fairly common in commercial plum plantations. About the same time that the underground lesions were discovered, typical Wither Tip symptoms on the above-ground parts were seen on some of the plum stools. Thus, of six Mariana stools growing on the Station at that time, one had two shoots with Wither Tip and another had four, the other stools being free from disease. What was evidently an early stage in such infection was shown by a shoot bearing a leaf which was blackened towards the base of the lamina but the infection had not yet extended into the axis of the shoot. These withered tips bore pustules of the Monilia stage of *S. laxa* on the leaves and on the shoot lesions. Typical Wither Tip was found also on a stool of Broad Leaved Mussel and on one of Pershore.

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* Until recently the Brown Rot fungus referred to here as *Sclerotinia laxa* was usually known as *S. cinerea* (*Monilia cinerea*). For a discussion on the reason for the change in nomenclature see *The Brown Rot Diseases of Fruit Trees*, Bull. No. 88 of the Ministry of Agriculture, Jan. 1935.

As shown in the experiments described below, both conditions, Wither Tip and Shoot Wilt of stool shoots, can be reproduced by infecting leaves with this fungus, the difference in symptoms depending on the situation of the infected leaves on the shoots. In Wither Tip, infection occurs on the upper leaves ; only the terminal portion of the shoot withers and the infected parts are above ground. In the Shoot Wilt condition, as here described, infection begins in leaves that are produced just above the level of the soil and are later covered with soil ; in this case the whole of the shoot, except perhaps a short portion at the extreme lower end, is destroyed.

INOCULATION EXPERIMENTS.

In May 1925 an inoculation experiment was carried out on a small layer plot in the open, eight shoots being inoculated by placing conidia of *Sclerotinia laxa* in drops of water on the lower leaves, which were covered with soil the next day. About a month later, two of the inoculated shoots were dead, and from one of these *S. laxa* was re-isolated, the other shoot being too decayed for isolation purposes. Of forty-eight shoots which were not inoculated, two also were dead. Although the ratio of infected shoots to the healthy ones was higher in the inoculated (2:6) than in the control shoots (2:46), the numbers were so low that the results, though suggestive, could not be considered as conclusive evidence that the fungus was the cause of the wilting. A similar experiment carried out the following year also gave inconclusive results.

It was then resolved to raise stools in pots so that the experiments could be carried out under greenhouse conditions. Rooted shoots were planted singly in seven-inch pots and the shoots cut back to soil level each year, to simulate the operation carried out in the field. The resulting stools, transplanted to twelve-inch pots, became established (each producing five to eight strong shoots) and ready for inoculation by the summer of 1933.* In the meantime, inoculations were made on leaves of aerial plum shoots in order to obtain some information regarding the mode of infection and the rate at which infection extends from a leaf into the axis of the shoot and through the tissues of the shoot to cause wilting.

INOCULATION OF LEAVES ON AERIAL SHOOTS.

Two days before they were inoculated, the trees were placed in an inoculation chamber to maintain a moist atmosphere. The inoculations were carried out by removing conidia of the fungus from a pure culture on glycerinated potato and floating them on sterilized water in a watchglass ; drops containing conidia were transferred to the young leaves by means of a platinum wire loop.

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* The writer wishes to express his indebtedness to Mr. A. Tidy, greenhouse assistant, for raising and attending to these plants before and during the course of the experiment.

Repeated attempts to induce infection on uninjured leaves on shoots of young trees, using varieties Victoria and Brompton (the latter being a plum stock commonly propagated and one of those from which *Sclerotinia laxa* had been isolated) gave negative results.

When, however, the leaves were injured by puncturing before inoculation with conidia, infection readily occurred.

In an experiment in 1930 four similar trees growing under the same conditions were used. On each tree six shoots were selected, and one leaf, towards the apex of each shoot, was marked (by a loop of cotton) for inoculation. On this leaf a drop of sterile water (on control trees) or a drop of water + conidia was placed. On two of the trees each spot on which a drop of water was to be placed was punctured with a sterile needle (two punctures about 1 mm. apart at each spot).

Thus the four trees had the following treatment:

Tree 1: leaves uninjured, inoculated with drops of water + conidia.

Tree 2: leaves uninjured, drops of sterile water only applied.

Tree 3: leaves punctured, inoculated as in Tree 1.

Tree 4: leaves punctured, drops of sterile water only applied.

The inoculations were made on May 22nd. No infection occurred on Trees 1, 2 and 4. On Tree No. 3 five of the six inoculated leaves became infected and the course of the infection on this tree was as follows:

May 24th. The inoculated leaf on each of five shoots was becoming "water-soaked" immediately around the punctures.

May 26th. The "water-soaked" areas were now 3-10 mm. in diameter. On two leaves (shoots No. 4 and No. 5) infection had extended to the base of the lamina which was beginning to droop in consequence.

May 27th. On those two leaves infection had reached to the base of the petiole, on the other three (shoots Nos. 1, 2 and 3) to the base of the lamina and the leaves were drooping.

May 28th. On shoots 4 and 5 the infection had extended into the axis of the shoots, upwards from the node for 5 mm., and 10 mm. downwards. On shoots 1, 2 and 3 infection had just reached the base of the petiole.

May 29th. A lesion was now present on the axis of each shoot extending upwards and downwards from the point of attachment of the infected leaf, as shown in Fig. 2.

No. 1: 5 mm. upwards, 8 mm. downwards, half girdling the shoot.
No. 2: 5 ,, ,, 10 ,, ,, ,, ,, ,, ,,
No. 3: 3 ,, ,, 6 ,, ,, completely girdling the shoot.
No. 4: 10 ,, ,, 20 ,, ,, ,, ,, ,, ,,
No. 5: 12 ,, ,, 18 ,, ,, ,, ,, ,, ,,

The shoots 3, 4 and 5 were now wilting from above the lesions, and Monilia pustules were present on all the infected leaves.

By May 29th, the eighth day after inoculation, all the five shoots had collapsed above the lesions. Thus typical Wither Tip condition of the shoots occurred within eight days after inoculating the leaves.

INOCULATION OF STOOL SHOOTS.

In this experiment leaves were inoculated on shoots growing from buds near ground level on stools in twelve-inch pots as already indicated. Some leaves were inoculated after puncturing, others without wounding. In the former, one leaf on each shoot was punctured at four points, two on each side of the midrib ; a drop of water containing conidia was placed on each spot and a sterilized needle pushed through the drop and through the leaf lamina. On those inoculated without wounding four drops containing conidia were placed on the lower surface of the leaves. On control plants drops of sterile distilled water only were applied to certain leaves ; some of these leaves were punctured, others left uninjured.

The plants were kept in an unheated greenhouse throughout the experiment. The leaves were inoculated on April 28th (1933) when the shoots were five to seven inches high. Eleven plants were available and they were treated as follows:

No. 1: control, 6 shoots, leaf punctured.
No. 2: ,, 4 ,, ,, ,,
No. 3: ,, 3 ,, ,, not punctured.
No. 4: ,, 7 ,, ,, ,, ,,
No. 5: inoculated, 5 shoots, leaf not punctured.
No. 6: ,, 3 ,, ,, ,, ,,
No. 7: ,, 5 ,, ,, ,, ,,
No. 8: ,, 4 ,, ,, punctured.
No. 9: ,, 4 ,, ,, ,,
No. 10: ,, 3 ,, ,, ,,
No. 11: ,, 3 ,, ,, ,,

Thus: 13 shoots were inoculated without puncturing,

14 were inoculated at punctures on the leaves,

20 were treated with water only, as controls, while on certain plants some shoots were left untreated and these also served as controls.

The treated shoots on a plant were marked by a loop of cotton tied loosely round the upper end of each.

Partially sterilized soil (steamed for one hour) was then added to the pots so that the inoculated leaves were covered with soil. In order to facilitate the earthing-up a piece of sheet zinc was placed round the inner side of each pot so as to form a false rim, thus increasing the height of the sides by about five inches.

The first evidence that infection had occurred was seen on May 8th (only ten days after inoculation), when one shoot on each of three plants, in the inoculated and punctured series, began to wilt. Eventually wilting was observed on five of the inoculated plants, as follows:

No. 5: Three shoots on May 16th.

No. 8: One shoot on May 15th.

No. 9: One on May 8th, another on the 9th, and a third on the 12th.

No. 10: One on May 8th.

No. 11: One each day, May 8th, 15th, 16th, 20th.

After May 20th no further shoots became affected. The wilted shoots gradually withered, turned brown and resembled those naturally infected in the nursery (see Fig. 5).

On plant No. 11 not only did each inoculated shoot become infected but one of those not inoculated also succumbed. This probably became infected by spores carried from the inoculated shoots when the soil was applied.

On May 30th five of the wilted shoots were removed from the plants and examined. The lesions were from 12 to 19 cm. long. Thus in four and a half weeks the fungus had destroyed the inoculated leaf, extended into the shoot and then destroyed the tissues upwards and downwards for a distance, in some cases, of about 10 cm. Transverse sections showed mycelium present in the cortex, the xylem and particularly the pith. A portion of each of these five shoots in the infected region was cleansed by sponging with cotton wool moistened with alcohol. The cortex was scraped off and transverse sections cut, or sections were cut first and then the cortex teased away. The decorticated sections were washed in sterile water and placed on agar plates. All the plates gave rise to growth typical of *Sclerotinia laxa* and transfers to glycerinated potato resulted in the production of the grey conidial stage of this fungus.

At the end of the growing season the shoots were uncovered down to the crown and all were cut away. The soil removed was placed in other pots and occasionally watered to prevent drying out. In the following spring, when the young shoots were a few inches high, the soil was replaced so that each pot again received the soil that had been removed in winter. The object was to ascertain whether the soil taken from pots containing infected shoots was capable of carrying infection to the shoots the following year. No shoots wilted, however, in any of the pots so there was no evidence that the soil itself becomes a source of infection. Moreover, the stools that bore infected shoots in 1933 were quite free from the disease the following year. It would seem then that the infection is localized in the individual shoots and is not carried over from one season to the next in the stool, or in the soil.

DISCUSSION.

The experiment just described indicates that the symptoms as seen in the nursery rows can be reproduced by inoculating plum leaves with conidia of *Sclerotinia laxa* before they are covered with soil, particularly if the leaves are injured. Infection, however, did occur in one plant on which the leaves were not deliberately injured, but the possibility that injury occurs in the process of earthing-up, or by insect larvae in the soil, cannot be ignored. The earlier experiments showed that infection can be induced fairly rapidly if the leaves are injured. Failure to obtain infection through uninjured leaves, though not easily obtained under experimental conditions, appears not to be impossible for Drummond (1) has recently reported success in a few cases.

The outbreak of the disease in the nursery may perhaps be partly explained by the proximity of the plot to another (about eighty yards away) on which standard plum trees were growing, the latter probably providing sources of infection. The weather conditions in the spring of 1924 were doubtless also an important factor. During that year the number of hours of sunshine during April, May and June was well below the average for the preceding nine years, while the rainfall in April and June was almost double, and that of May only very slightly below the average. The soil moisture, abnormally high for the time of the year, must have been particularly favourable for infection by the Brown Rot fungus.

In the following year (1925) the same disease appeared again on the same plot but with less intensity, and in recent years it has been almost negligible in the stool and layer rows at East Malling. The main stool beds are now at some considerable distance from the main plots of plum trees, and the weather conditions during the infection period in the last ten years have generally been less conducive to infection than in 1924.

CONTROL.

It would appear then that the wilt disease of stool and layer shoots in plum nurseries is chiefly brought about by spores of *Sclerotinia laxa* falling on leaves that are subsequently covered with soil during the normal earthing-up of nursery practice. The fungus is a common parasite infecting the flowers and fruit of plum and cherry trees, and on plum trees it also infects the leaves and young shoots. Such infected parts therefore serve as sources of infection and, in order to avoid an attack of the disease here described, stool and layer beds of plum stocks should not be laid out in the vicinity of plum or cherry plantations unless precautions are taken to control the disease on such trees.

Fungal fructifications have not been found on the underground lesions, so the dead shoots are not themselves sources of infection. However, if lesions occur on the parts above ground, Monilia fructifications may develop on the dead tips and shed their spores into the air. Thus shoots showing the Wither Tip condition should be cut back or pulled out.

SUMMARY.

A Shoot Wilt in stools and layer rows of plum stocks was observed in the summer of 1924 at East Malling.

Lesions containing fungal mycelium were found on the underground parts of the affected shoots, and *Sclerotinia laxa* was isolated from the tissues of the lesions.

Typical Wither Tip, caused by the same fungus, was also found on certain stools on the same plot.

Experiments have shown that both conditions can be induced by inoculating punctured leaves with conidia of the fungus.

Shoot Wilt was produced on stools in pots by inoculating leaves and then adding soil to the pots to cover such inoculated leaves.

It is recommended that stool and layer beds of plum stocks be laid out as far removed from established plum trees as possible. Any shoots showing Wither Tip should be cut back or pulled up.

REFERENCES.

(1) *Drummond, R.* Notes on the Wither Tip Diseases of Plums caused by *Sclerotinia cinerea* and on the Blackening of Apples caused by *Sclerotinia fructigena.* Journ. Pom. & Hort. Sci., 1934, **12**, 105-9.

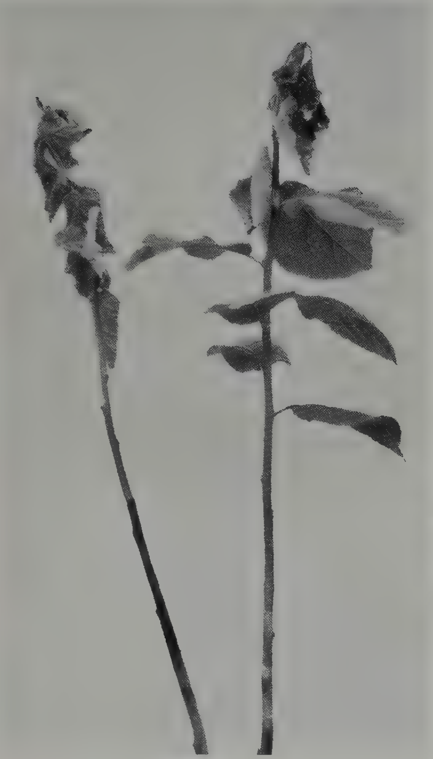

FIG. 1.
Stool shoots of Brompton. On the left Shoot Wilt, showing an underground lesion; on the right Wither Tip.

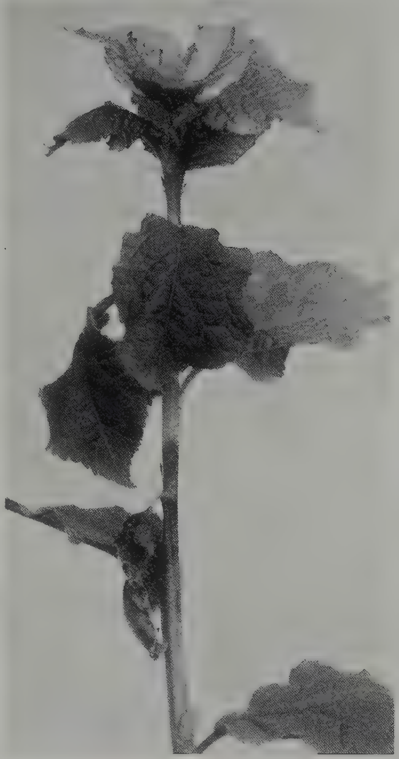

FIG. 2.
Brompton shoot seven days after inoculating a leaf with conidia of *Sclerotinia laxa*. Note the dead leaf, and lesion on the axis of the shoot.

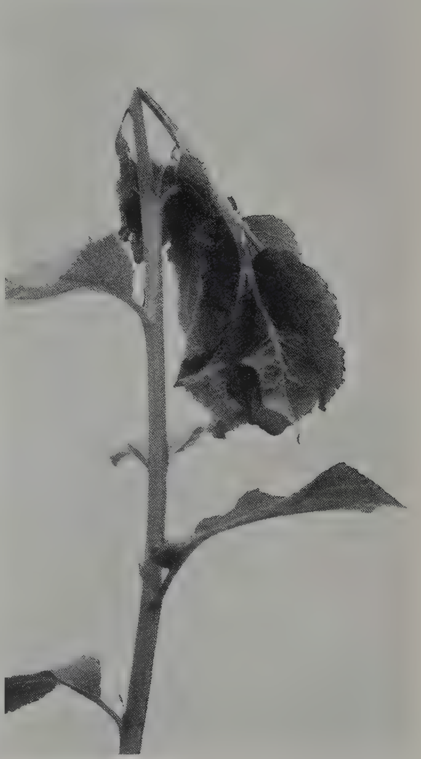

FIG. 3.
The same shoot as in Fig. 2 but a day later.

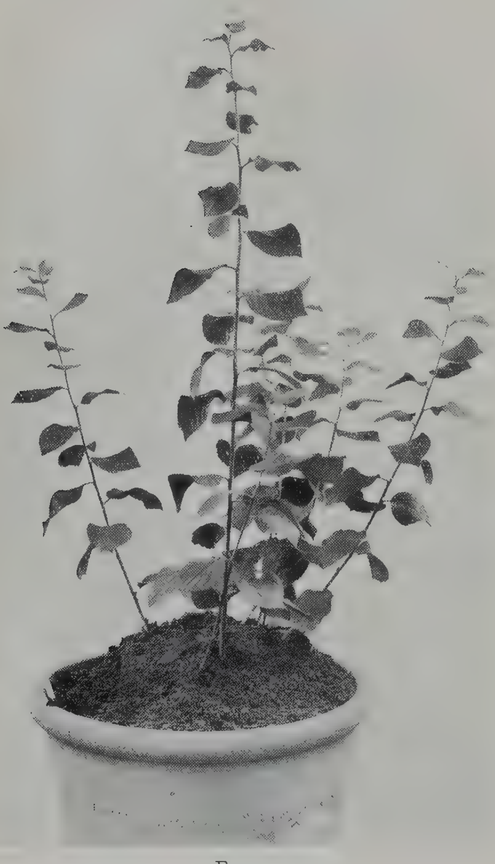

FIG. 4.

A Brompton stool; control plant, leaves punctured but not inoculated.

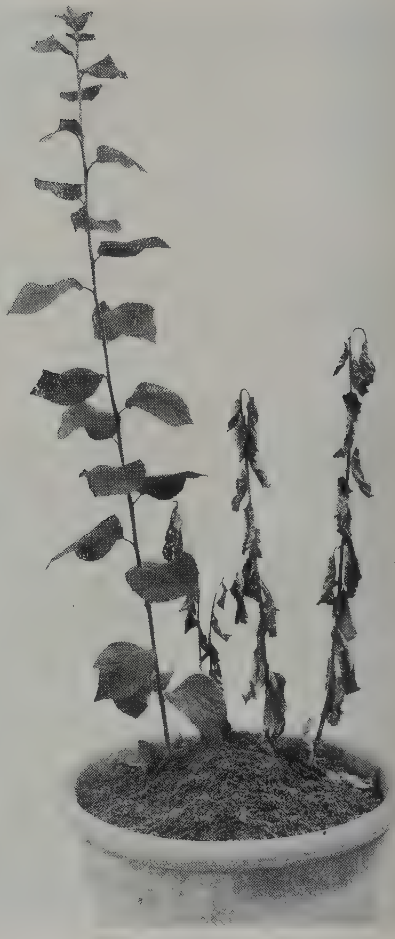

FIG. 5.

A Brompton stool on which three shoots were each inoculated at punctures on a leaf, before earthing-up. All three shoots wilted and one other shoot also became infected; result four weeks after inoculation.

(2) *Wormald, H.* A "Wither Tip" of Plum Trees. Ann. Appl. Biol., 1918, **5**, 28-59.

(3) ———. Further Studies of the Brown Rot Fungi. I. A Shoot Wilt and Canker of Plum Trees caused by *Sclerotinia cinerea*. Ann. Bot., 1922, **36**, 305-20.

(4) ———. Notes on Plant Diseases in 1924. Ann. Rpt. East Malling Res. Sta. for 1924 (1925), 112.

(5) ———. The Brown Rot Diseases of Fruit Trees. Min. of Agric. and Fisheries, Bull. **88**, 1935, 32.

(*Received* 21/1/35.)

BOOK REVIEWS

THE GENETICS OF GARDEN PLANTS. By M. B. CRANE and W. J. C. LAWRENCE. (Macmillan & Co. Ltd. 1934. 10s. 6d. net.)

During the last twenty years the science of genetics, assisted by cytology, has moved rapidly forward, both in matters of principle and in solving particular problems. This book tells no continuous story, but deals with the special subject of garden plants, which have attracted the attention of many genetical workers, partly because of their value to man, and partly because the practical breeder, attempting to produce better varieties, has come across many interesting genetical problems. The knowledge now gained should be useful to all who are interested in the breeding of these plants, and our gratitude is due to Mr. Crane and Mr. Lawrence, who are exceptionally well qualified to write on the subject, for assembling it.

Most garden plants have been hybridized for many years, and their genetics is complicated. The authors have accordingly devoted the first forty or so pages of the book to introducing and explaining the genetical principles involved. This should assist those who already have some knowledge of genetics and who wish to understand the special problems, many of them highly technical, that occur in garden plants ; but those without special knowledge will often find the book difficult. After this introduction come a summary of genetical knowledge in the different species. In some of these, such as the onion and lettuce, very little is yet known. But in many of them, such as some of the fruits, and often through the work of the authors themselves, a great deal of interesting information is available. Separate chapters are given to topics of special interest such as bud-sports, incompatibility and sterility. Here we may learn when and why it may be necessary to interplant different varieties of the same fruit ; why some varieties of apple, though excellent themselves, give poor results as parents for breeding new varieties ; and so on. The book is well illustrated ; and, as might have been expected from these authors, is full of interesting items of information about the plants it deals with. A.E.W.

SCIENTIFIC HORTICULTURE (formerly the H.E.A. Year Book): THE JOURNAL OF THE HORTICULTURAL EDUCATION ASSOCIATION. Vol. III, pp. xliv. + 228, figs. 33. 1935. (Published by the Horticultural Education Association. Hon. Editor: R. T. Pearl, South-Eastern Agricultural College, Wye, Kent. Price 3s. 6d. net (postage 5d. extra).)

Vol. III of the well-known " H.E.A. Year Book " comes out under its new title of " Scientific Horticulture " and thus marks a recognition not only of the rapid strides that horticultural research has made in the past decade but also of the essential place of research in horticultural practice. The first paper is Dr. Wallace's Presidential Address on " Science and Fruit Growing " which is a brief commentary on the additions to pomological knowledge made at Woburn, Long Ashton and East Malling. Whether the implementing of his suggestions of the establishment of " a University Chair or Lectureship in

Pomology" and "the foundation of an Honours School in Pomology" at one of our Universities is feasible or even desirable, is a matter for discussion. There follows a survey of "Commercial Horticulture in Northern Ireland" by W. J. Megaw and E. E. Skillman and it is interesting to note that export of apples has risen from 6,000 cwt. in 1924 to 106,392 in 1933 and also that whilst in 1923-24 school gardening was taught in only sixteen schools, by 1933-34 the number had risen to one hundred and seventy-one. A useful article by J. Turnbull on "Fruit Tree Spraying Equipment" opens with "The selection of suitable spraying equipment has more to do with the control of pests and diseases than is generally realized" and it is to be hoped that this paper will be widely read. N. B. Bagenal and R. T. Pearl report on the 1934 R.H.S. Conference on Apples and Pears and B. S. Furneaux contributes an interesting paper on the somewhat neglected problems of "Selection of Soils for Dessert Apple Growing". In 1934 East Malling came of age and it was something of a shock for many of us to realize that the immense body of knowledge which this Station has contributed to Pomology, and the immense influence it has had on orchard practice, have been acquired in twenty-one years of which four were war years. Few research stations in the world have justified themselves so abundantly as has East Malling, and the paper by R. T. Pearl and R. Hart on "Twenty-one Years' Fruit Research at East Malling" presents a fascinating record of progress. There is a short paper by Sir Albert Howard on "The Waste Products of Horticulture" and their utilization as humus by means of the well-known Indore process, and one by M. D. Glynne and H. V. Garner on "Research at Rothamsted of Importance in Horticulture".

The greater part of the issue consists of Bulletin XLVII of Reading University, being "Papers Read at the Second Revision Course in Horticulture", September 1934, and this, consisting of some one hundred and forty-four pages, is inserted in the middle of the paper by Pearl and Hart which it breaks in an unpleasant and unnecessary manner. The course was organized by Professor Stoughton and the bulletin contains sixteen useful papers dealing with various problems of vegetable and bulb cultivation. The first seven papers deal with vegetables and the opening paper by H. V. Taylor on "The Vegetable Industry To-day: Home Supplies and Imports" is a valuable and timely statement which brings home to one the enormous importance of the horticultural industry in the economic life of the nation. If statesmen and agricultural polemicists could be made to realize that the value of Brassica crops alone is nearly twice that of the wheat crop, perhaps the farming industry in this country might be seen in truer perspective. Hoare contributes a paper on "The Manurial Problem in Relation to Vegetable Production" pointing out how little we know of these matters and how greatly research is needed. There follows a valuable account by F. A. Secrett of "Irrigation of Vegetable Crops" in which details are given of the methods which the author has so brilliantly applied on his own farms. S. J. Wright contributes a paper on "Machinery in Vegetable Production" which is both suggestive and full of practical knowledge and experience. The canning industry has had an astonishingly rapid growth in this country, and in 1933 the pea pack was approximately fifteen million cans. The industry teems with problems and a good account of "Vegetables in Relation to the

Canner " is given by F. Hirst. L. Ogilvie and B. O. Mulligan describe recent Long Ashton work on " Vegetable Diseases ", and H. W. Miles gives an account of his work on " The Control of Insect Pests of Vegetable Crops ", strongly recommending repellant dusts. There follow three papers on glass-house crops, two by W. Corbett on " Points in the Cultivation of Tomatoes, Cucumbers and Lettuces in Glasshouses ", and " The Improvement of Glass-house Soils " and one by C. E. Hudson on " The Heating of Commercial Glasshouses ", and all contain much valuable knowledge and experience. The remaining six papers deal with bulbs, the first paper being by W. H. Waldock on the " Commercial Aspects of the Bulb Industry ". It is interesting to learn that " the English grown narcissus bulb is always more dependable and satisfactory than the imported Dutch article " but that it is doubtful " whether our present sun-drying system of harvesting tulips will ever yield the consistency of results attained by the highly perfected heat-drying system practised in Holland ". J. C. Wallace and D. E. Horton write on " Some Factors in Commercial Bulb Production " giving the sound advice that " Bulbs should be treated more like eggs than potatoes ". A paper by G. W. Gibson on " Some Observations on the Manuring of Bulbs " points out the great need for research on these problems. Papers by A. Beaumont and by W. E. H. Hodson deal respectively with " Diseases of Narcissi and Tulips " and the " Control of Insect Pests of Bulbs " and both contain the results of much first-hand experience. The final paper is a synthesis of an open discussion on " The Production of Flowers from Bulbs " and illustrates many of the difficult problems involved. The whole series of papers forms an admirable cross-section of our present knowledge of vegetable and bulb cultivation and shows clearly what ample justification there was for the change in the title of the publication.

The work is well printed and excellently illustrated and, as from one editor to another, I should like to congratulate Mr. Pearl on the way in which he has produced a publication of which the Horticultural Education Association may well be proud.

W.B.B.

STATISTICAL METHODS FOR RESEARCH WORKERS. By R. A. FISHER. (Oliver & Boyd. 1934. 5th edition. 15s. net.)

Ten years ago the appearance of Professor Fisher's book started something like a revolution in all branches of biological investigations. In his preface to the first edition, Professor Fisher says: " Little experience is sufficient to show that the traditional machinery of statistical processes is wholly unsuited to the needs of practical research. Not only does it take a cannon to shoot a sparrow, but it misses the sparrow! " To-day it is difficult to realize how inadequate those processes were, so rapid have been the advances since that time.

A comparison of the latest edition with the first gives some idea of ever widening application of statistical methods in research and much of this has been made possible by the works of Professor Fisher and his colleagues in the Statistical Department at Rothamsted.

In the present edition, various sections of the book are expanded to bring them up to date. In particular, the section on the analysis of covariance is enlarged and the proper treatment of tests of significance explained.

T.N.H.

THE MORPHOLOGY, PHYSIOLOGY AND MODE OF PARASITISM OF A SPECIES OF *CHALAROPSIS* INFECTING NURSERY WALNUT TREES*

By **JOYCE B. HAMOND, Ph.D.**
Research Station, East Malling, Kent

I. Introduction.

During the last few years, methods of propagating the walnut have been under investigation at East Malling Research Station, with the object of raising trees of good, named varieties (9). During the course of this work certain diseases appeared, and of these, two were adversely affecting the propagation work, namely, a bacterial disease, and a disease affecting young grafted plants. The bacterial trouble proved to be Walnut Blight caused by *Pseudomonas juglandis* (10), but the "graft disease", appeared to be one not previously described.

In order to understand the relation that this disease bears to walnut cultivation, a description of the methods of propagation and grafting, as practised at East Malling, is necessary (6). At present one-year-old *Juglans nigra* seedlings are used as stocks. These are lifted from the open nursery in November, potted, and kept in a cool greenhouse until February when they are moved into the propagation house preparatory to grafting.

During the winter, scion-wood is collected from trees of good varieties, and cut into convenient lengths. Before grafting, the stocks are cut down to about three inches above the soil level in the pots, and the scion is actually inserted on the tap-root or just above it, a double whip-and-tongue graft being used. The pots are plunged into coconut fibre in closed frames, where the atmosphere is kept moist, and there is a bottom heat of 60°-70° F. After about three weeks the grafted walnuts are removed from the closed frames. In normal cases a union will have taken place during this time and new growth will have appeared. Using good, well-ripened graftwood about 80-90 per cent. success is usually obtained by this method.

In the spring of 1930 there was an almost complete failure of the walnut grafts. About three weeks after grafting, the buds, instead of breaking into new growth, began to fall off. On examining these grafts it was seen that in many cases a discoloration had appeared at the junction between the stocks and the scions, and was extending upwards into the scions. On untying the diseased grafts, a layer of soot-like powder was seen covering the cut surfaces of the stocks and scions. This was found to consist of a mass of dark brown, ovoid spores, measuring about $17\mu \times 13\mu$. The spores formed a loose mass and it was not clear how they were produced. The fungus was therefore isolated in pure culture, as a means, in the first place, of identifying it.

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* The present paper is an abridgment of a Thesis (presented June 1934) approved for the Degree of Doctor of Philosophy in the University of London.

These spores germinated readily in hanging-drops of carrot extract, and each gave rise to smaller, hyaline spores produced within a tubular conidiophore. It was apparent, therefore, that the fungus had two forms of spores: macrospores, large, dark and thick-walled, and microspores, small, cylindrical and produced endogenously. These two spore forms at once suggested *Thielaviopsis*. Later, however, it was found that the macrospores were borne singly and terminally, not in chains as in *Thielaviopsis*. This character indicated that the walnut graft fungus must be referred to the genus *Chalaropsis* of Peyronel.

In the summer of 1931 it was noticed that a young, grafted walnut tree which had been planted in a permanent position at East Malling during the previous winter, was bearing small, yellowish leaves on slender, poorly-ripened shoots, and appeared to be generally unhealthy. In the spring of 1932, this tree failed to come into leaf and was dug up during March. It was found that the tree had produced hardly any new roots, and that most of the tap-root was black and disintegrating. Dark brown spores similar to those on the diseased grafts were present in abundance. In this case, infection had apparently started at the cut surface of the tap-root, which had been trimmed before the stock was potted up. The fungus had spread throughout most of the root system, but the union between the stock and the graft was healthy. The cortex of the trunk of the young tree was discoloured, but the fungus was not found in the trunk. The fungus was isolated from the roots of this tree, for comparison in culture with that obtained from the diseased grafts.

On finding the fungus apparently infecting the tap-root of a walnut tree, seedling stocks, which had been potted up in November 1931, but had not been grafted in the spring of 1932, were examined for root infections. In several cases a black layer of spores was found at the cut surface of the tap-roots. The lesions, at that time, were quite superficial. Other stocks which had been grafted in the spring of 1932, but which had failed to unite with the grafts, were examined in April 1932. The fungus was present at the union except in rare cases, where the use of poorly-ripened scion-wood accounted for the failures. Sometimes the fungus was also present on the tap-root; frequently, however, callus tissue had formed at the cut surface of the tap-root, and new fibrous roots were being produced normally. These observations indicate that lesions at the union between stock and scion, and the lesions on the roots, are quite independent of each other, and that, when conditions are favourable, the fungus is liable to appear at any cut surface of walnut root or shoot. This is borne out by the fact that the fungus appeared on a tap-root of a stock which had accidentally been split throughout its length when it was lifted from the open ground. In this case, a white powder was noticed on the part of the split root which was above the soil. This was found to consist of a mass of hyaline endospores, which are always produced readily when infected tissues are exposed to a warm, moist atmosphere. Macrospores were present within the upper part of the tap-root, and also on cut surfaces below the soil.

In the spring of 1932, several layers that had been planted in the autumn of 1931 and had died, were examined. Here again a cut surface had been exposed in the soil and had been attacked by the same fungus. The roots were black and disintegrating. A fresh isolation was made from this source, but no constant cultural or morphological difference could be found between this strain and that obtained from the roots of the dead tree.

In the autumn of 1930, a *Chalaropsis* culture was received from Dr. A. Smith, of the Plant Pathological Laboratory at Harpenden. The fungus had been found on diseased carrots, but was not regarded as a serious parasite. It was very similar to the fungus isolated from walnut, endospores and macrospores being produced in the same manner. The macrospores, however, were more spherical in shape, and the endospores appeared olive green in mass, instead of white.

In July 1933, another *Chalaropsis* culture was obtained from Mr. E. W. Mason, of the Imperial Mycological Institute, Kew. The fungus had been found by Dr. I. Reichert on peach seedlings imported into Palestine from France; specimens had been sent to the Imperial Bureau of Mycology and Mr. Mason had isolated the *Chalaropsis* from them. In its salient morphological characters this strain was indistinguishable from the walnut fungi, but it was very prone to sectoring in plate cultures, and could always be distinguished by this character.

The four strains indicated above have been used throughout this investigation and will be referred to as: (a) Walnut Graft fungus, (b) Walnut Root fungus, (c) Carrot fungus, (d) Peach fungus.

II. MORPHOLOGY OF THE FUNGUS.

The four strains of *Chalaropsis* which have been isolated from a walnut graft, walnut root, carrot and peach respectively, all bear a general morphological resemblance to one another when grown in culture.

A. *Mycelium.*

When the fungus is growing freely, only a small amount of mycelium is present, and this is chiefly in the form of endoconidiophores connected by short strands of septate, vegetative hyphae. The macroconidia are frequently sessile or borne on unicellular conidiophores arising from the base of the endoconidiophores (Fig. 1). Each endoconidiophore consists of 1-4 basal cells, terminating in a tapering endoconidial cell. The conidiophores are hyaline at first, but after a few days the basal cells assume an olive-green colour, which decreases in intensity throughout the length of the conidiophore until the hyaline endoconidial cell is reached. The basal cell of the conidiophore is generally swollen and curved where it is joined on to the vegetative cell from which it arises, resulting in a characteristic " crook ".

The vegetative cells and the cells of the endoconidiophores are at first full of protoplasm which contains a few scattered guttules. After about a fortnight, vacuoles appear in the protoplasm and the guttules are smaller and more numerous, giving a granular appearance. The dimensions of the cells are $13{\cdot}5$-$45{\cdot}0\mu \times 2{\cdot}0$-$6{\cdot}5\mu$. The vegetative cells and the macroconidiophores are similar in size and appearance to the basal cells of the endoconidiophores.

The carrot strain differs from the rest in certain characters. The olive-green colour of the mycelium appears after the cultures have been growing for two days only, instead of after a week or ten days, as in the case of the other strains. This green coloration is much more pronounced in the case of the carrot fungus than in the other three strains. The basal cells of the endoconidiophores remain unswollen and usually show no crook where they join the vegetative mycelium (Fig. 2). When they are growing freely, all these fungi give off a pineapple odour ; this decreases in intensity when the cultures are about a fortnight old and are growing more slowly. The pineapple odour is always stronger in the case of the carrot fungus than in comparable cultures of the other strains. The carrot strain also differs from the rest in the shape and size of its macrospores ; this is referred to later.

When any of these strains of *Chalaropsis* are cultured on a medium on which they grow slowly, the mycelium presents quite a different appearance from the normal growth which has just been described. At first, normal endoconidiophores are produced, but after about four days, very thin, sparsely septate, sparsely-branched, vegetative mycelium appears in profusion beyond the centre of the culture which is occupied by normal mycelium. This abnormal mycelium has very thin walls, and the cells are often vacuolated. After about a week some of the cells swell up to produce irregular, intercalary swellings. As the culture gets older, the mycelium darkens and guttules appear in the protoplasm lining to the cells. These guttules occur most freely in poorly-growing cultures of the walnut root and carrot strains, imparting a yellowish green colour to these cultures. The characteristic intercalary swellings associated with this abnormal mycelium are least pronounced in the case of the carrot strain, where these hyphal branches sometimes terminate in macrospores.

Macrospores have never been found in association with this abnormal mycelium in other strains. As this type of mycelium is always associated with unfavourable conditions, it will be known as "deficiency" mycelium in this paper. The cells measure 15·0-70·0μ × 1·0-5·5μ. The widest cells, and therefore those most approaching the normal, are found in the carrot fungus. Abundant deficiency mycelium is found beneath the surface of the culture agar where the fungus is growing badly. In the carrot strain, macrospores are sometimes borne on the tips of these hyphal branches, but macrospores have not been found in association with such hyphae in other strains. When the fungus is growing well, abundant mycelium is also found beneath the surface of the agar. This mycelium is similar to deficiency mycelium in its habit of growth and cell measurements, but intercalary swellings are absent. Moreover, where the fungus is growing luxuriantly, macrospores are frequently borne terminally on the hyphae below the surface of the agar in every strain, and in old cultures they are present in sufficient number to impart a dark colour to the media.

B. *Conidia.*

1. *Endospores.* These spores are always produced in abundance when the fungus is growing freely in the presence of air and moisture, either on a living host or saprophytically in culture. They are produced one by one within the upper part of the endoconidiophore, and pushed out of the apex of the endoconidial cell. Frequently, a dozen or more spores may remain attached in a chain, when they are being produced quickly. Each spore is hyaline when produced and is cylindrical with rounded ends. Small guttules occur at the extremities of the spores and there is a vacuole in the centre. The dimensions of the endoconidia of the four strains are given in Table I. The abnormally long spores which may occur in culture are not found when the fungus is growing as a parasite (Table II).

The endospores of the carrot fungus are usually slightly shorter than those of the other strains and they are pale olive-green in mass instead of hyaline.

TABLE I.

Dimensions of endospores.

Strain.	Endospore lengths.		Endospore widths.	
	Extreme Measurements.	Average.	Extreme Measurements.	Average.
Graft	12·0–40·0μ	16·3μ	2·0–4·5μ	3·2μ
Root	11·0–30·0μ	17·1μ	2·0–4·5μ	3·1μ
Carrot	9·0–24·0μ	14·2μ	3·0–6·0μ	3·6μ
Peach	12·0–32·0μ	19·6μ	3·0–5·5μ	4·4μ

TABLE II.

Dimensions of endospores on Walnut host.

Strain.	Endospore lengths.		Endospore widths.	
	Extreme Measurements.	Average.	Extreme Measurements.	Average.
Graft	7·5–16·5μ	12·4μ	3·5–5·5μ	4·5μ
Root	9·0–19·0μ	13·0μ	3·0–5·0μ	4·0μ

The behaviour of the endospores in culture varies according to whether the fungus is growing luxuriantly or feebly. If the fungus is growing well, the endospores swell up and become ovoid or pyriform after about a week, preparatory to germination. Frequently, chains of spores do not disintegrate, and some or all the spores in the chain may become swollen. These swollen spores are vacuolated and contain several large guttules. The protoplasm becomes olive-green in colour in older spores. These swollen spores rarely germinate, and if they do so, a short germ-tube only is produced. In these cultures which are growing well, new endospores are continually being produced as long as nutrient material is available.

In cultures where the fungus is growing badly, the spores begin to swell up after about three days, and the contents become more granular in these spores than in those of cultures where the fungus is growing well. These swollen spores often germinate and produce septate germ-tubes which may show intercalary swellings such as those associated with deficiency mycelium. After about a fortnight, endospores which have developed in a different way begin to appear in starved cultures. Here, instead of the whole spore becoming swollen, as is normally the case preparatory to germination, one side of the spore only becomes swollen, producing a body which is more or less spherical according to the stage of development it has reached, and at one side of which, the unswollen portion of the endospore can be distinguished. These spherical bodies have also been seen to germinate.

Throughout these observations on the development of endospores in culture, it has been noticed that those produced in cultures of the carrot fungus show least tendency to swell and finally to germinate. When this fungus is growing well, the endospores become full of guttules, but the vacuoles remain small, so that while the spores may become ovoid, large, pyriform and irregular spores are only seen very occasionally. The circular bodies observed in the other strains have rarely been seen in this strain. The spores in the strain isolated from walnut root have always shown a tendency to contain more granules and to germinate more rapidly than the spores in the other strains.

Endospores from the four strains of *Chalaropsis* were germinated in sterile water. The spores were taken from comparable, quickly-growing cultures in each case, and hanging-drop cultures set up. Short, non-septate germ-tubes were produced after twenty-four hours from the walnut and peach strains, and guttules from the spores were seen passing into the germ-tubes. Most of the endospores of the carrot strain had only swollen preparatory to germination. Several spores from the walnut root strain had produced germ-tubes which had developed immediately into conidiophores and were giving rise to further chains of spores.

After forty-eight hours, the hanging-drop cultures showed very little further change. After seventy-two hours, however, short vacuolated germ-tubes had appeared in the case of the carrot strain, and the germ-tubes had become septate and vacuolated in the other strains. Endospores were being produced in the case of the peach fungus.

When comparable endospores from the four strains were set up in hanging-drops of 2 per cent. carrot extract, septate germ-tubes were produced after twenty-four hours. In the case of the carrot strain, however, these germ-tubes were short and stubby. Endospores were again produced most readily in the case of the walnut root fungus, although the other strains produced them in three days. In every strain the mycelium became vacuolated after this time.

2. *Macrospores*. These spores are produced less readily in culture than the endoconidia. They may occur on short conidial branches, but they are frequently sessile. Each spore is borne singly and terminally, several usually occurring together in a sympodial arrangement. When the individual conidiophores are very short, the spores may be produced in a botryoid cluster. In the strain isolated from carrot, the macrospores tend to be borne on longer conidiophores, which are more scattered, instead of arising in compact groups, as in the case of the other strains. The spores mature in acropetal succession, the apical ones being hyaline, while those at the base gradually become dark brown. The young hyaline spores are thin-walled and contain few small guttules. As they mature, a thick, dark brown perispore develops and the spores become almost full of guttules which may finally coalesce into a few large globules.

The macrospores are typically ovoid. The strain isolated from carrot can always be recognized in culture by its macrospores which are almost circular in shape, the average dimensions being $12{\cdot}1\mu \times 12{\cdot}9\mu$ (Table III). Spores taken from naturally-infected walnut stocks have also been measured; these are found to be slightly larger and more irregular in shape than those obtained in culture, the irregularity being probably due to the pressure they receive from one another and from cell walls of the host, as they are present in larger numbers than when grown in culture.

TABLE III.

Dimensions of macrospores.

Strain.	Macrospore lengths.		Macrospore widths.	
	Extreme Measurements.	Average.	Extreme Measurements.	Average.
Graft	12·0–19·0μ	14·9μ	8·5–13·0μ	10·9μ
Root	14·0–19·5μ	17·4μ	9·5–14·0μ	12·1μ
Carrot	11·0–16·0μ	12·9μ	10·0–14·0μ	12·1μ
Peach	13·0–23·0μ	17·0μ	9·5–14·5μ	12·0μ

TABLE IV.

Dimensions of macrospores on Walnut host.

Strain.	Macrospore lengths.		Macrospore widths.	
	Extreme Measurements.	Average.	Extreme Measurements.	Average.
Graft	12·5–21·0μ	16·8μ	10·0–14·5μ	12·6μ
Root	13·5–22·0μ	17·0μ	9·0–16·0μ	12·8μ

Macrospores are produced most readily in culture by the fungus isolated from carrot. When this strain is growing well, they have been found after only two days. In the case of the fungi isolated from walnut root and peach, macrospores occur after about four days when the fungi are growing well. The fungus isolated from a walnut graft produces macrospores least readily; they usually appear after about seven days, when grown under the same conditions as the other strains. When these fungi are cultured on a medium on which they make poor growth, macrospores appear after about a week in the case of the carrot strain, but only after about a fortnight or three weeks in the case of the other strains.

The strain isolated from peach shows what appears to be a slight tendency to chain-formation in macrospores. In these cases a second, small, spore-like cell occurs immediately below a normal, terminal macrospore. This second spore has never been seen to develop any further, and is probably vestigial. These abnormal spores have been found most frequently beneath the surface of a medium on which the fungus grows readily. This feature is interesting, as it suggests the close relation between the genera *Chalaropsis* and *Thielaviopsis*.

Macrospores from freely-growing cultures of each of the four strains of *Chalaropsis* were used for germination studies. The macrospores were set up in hanging-drops of sterile water and examined at frequent intervals. A resting period of four days was required, after which short germ-tubes were put out by the spores taken from the carrot, root and peach cultures. After five days, a few macrospores from the walnut graft culture had germinated, while endospores were being directly produced from the germ-tubes of the other strains. These germ-tubes were unusual in that they developed immediately into short, hyaline conidiophores, instead of first producing branched, filamentous hyphae. The conidiophores became vacuolated during the production of endospores. Four to ten spores were usually formed.

Macrospores similar to those germinated in distilled water were set up in 2 per cent. carrot extract. In this medium, short germ-tubes were put out after twenty-four hours in the case of each strain except the walnut graft fungus. After forty-eight hours, the majority of macrospores from the walnut graft strain had germinated, while the germ-tubes of the macrospores from the other strains had become branched and septate. In seventy-two hours, chains of endospores were being produced from vacuolated germ-tubes of the walnut root, peach and carrot fungi (Fig. 3).

During the course of some later experiments, the method of production of endospores from macrospores germinating in sterile water was observed in detail, using macrospores from a naturally-infected walnut seedling stock. These macrospores were set up in a hanging-drop of distilled water, where they germinated more rapidly than the spores obtained in culture. A description of the process is as follows:

15 hours. A portion of the hanging-drop, which contained a number of germinating spores was placed on a glass slide and covered with a thin cover-slip, the edges of which were smeared with vaseline to prevent evaporation of the water. One spore, with a germ-tube about 25μ long was drawn and kept under observation (Fig. 4, No. 1).

18 hours. The germ-tube had now taken on the typical tapering shape of an endoconidiophore, and a vacuole had appeared (Fig. 4, No. 2).

18 hours 30 minutes. A transverse wall was laid down cutting off the apex of the germ-tube (Fig. 4, No. 3).

19 hours. A slight increase in length had taken place in the contents of the germ-tube between the macrospore and the newly-formed wall. This increase in length was brought about by an increase in the size of the vacuoles in this part of the tube. As a result of this increased pressure on the wall of the germ-tube, it had been split at the apex and the contents of the distal end of the tube, which had developed a wall, was protruding from the split sheath, as the first endospore.

19 hours 6 minutes. The newly-formed endospore had separated from the rest of the tube contents and was moving out of the tube. The contents of the tube were extending all the time, owing to vacuolation, and therefore the space between the endospore and the tube contents, was less than it would have been if the latter had remained stationary (Fig. 4, No. 4).

19 hours 11 minutes. The endospore, after travelling slowly out of the tube until only the distal, narrowing portion remained in the sheath, was shot out a distance of about 10μ (Fig. 4, No. 5). After the spore had been ejected, the apex of the tube was seen to be rounded in optical section, thus the diameter of the pore was narrower than the diameter of the rest of the tube. It is therefore presumed that the impetus for this rapid ejection of the endospore was supplied by pressure of the narrowing sheath on the spore.

19 hours 16 minutes. The tube contents had become more vacuolated, and had increased in length, until it had almost reached the opening of the sheath, after which a wall again appeared cutting off the second spore from the tube contents (Fig. 4, No. 6).

19 hours 52 minutes. The second endospore had commenced to separate from the tube contents (Fig. 4, No. 7).

During the germination of a macrospore, and the subsequent formation of endospores, the contents of the germ-tube gradually become more vacuolated, the final number of endospores depending, presumably, on the amount of material in the germ-tube, this being gradually used in endospore formation, and replaced by vacuoles.

The rapid production of chains of endospores in the presence of air and moisture, and their rapid germination, leading to the production of more endospores, seem to indicate that these spores serve to propagate the fungus in favourable conditions. When the fungus is growing freely, macrospores are also produced, although more slowly. These spores are not so dependent on air and moisture for their formation, and they are therefore found on plant tissues beneath the soil and in culture beneath the surface of solid agar. Macrospores, with their thick perispores, are obviously more resistant to desiccation and unfavourable conditions than endospores. Their primary function seems to be, therefore, to enable the fungus to survive unfavourable conditions.

III. CULTURAL RELATIONS.

The cultural relations of the four strains of *Chalaropsis* have been studied by growing them on various media, in culture tubes (slopes), on agar plates, and on pieces of plant material. Attention was given chiefly to type and rate of growth and colour changes.

When these strains of *Chalaropsis* are growing vigorously, as on carrot agar and on potato dextrose agar, endospores, for rapid dispersal of the fungus, are produced in abundance ; these are followed by macrospores to enable the fungus to survive when conditions become less favourable. These macrospores are formed most readily in the strain isolated from carrot, and least readily in the strain isolated from a walnut graft. A relatively small amount of mycelium is produced when these fungi are growing vigorously, and the endospores germinate very slowly. When these cultures are young the characteristic pineapple odour is strong, but this decreases as the cultures increase in age.

If conditions are unfavourable, as when these fungi are growing on malt agar or prune agar, endospores are produced less profusely, and they germinate quickly, developing long, branched germ-tubes. Alternatively, the endospores may swell up to form characteristic sphaeroidal spores, which eventually germinate. Macrospores may not be produced at all, or they may develop only after several weeks. Frequently these macrospores are light brown and never mature. Abnormal deficiency mycelium is produced in abundance when these fungi are making poor growth. When growing in unfavourable conditions, these strains of *Chalaropsis* tend to develop vacuolated swellings, both in connection with the mycelium and endospores. The pineapple odour is almost absent from these cultures.

When the four strains of *Chalaropsis* are grown on pieces of walnut stems and roots, growth is much more luxuriant on autoclaved material than on fresh material, but macrospores are produced more readily on fresh material. An interesting point which may have some significance, is that when the walnut graft fungus is cultured, macrospores are produced earlier on autoclaved stem than on autoclaved root material, while if the walnut root fungus is cultured, the reverse is true. This may indicate that the walnut graft fungus is more adapted for growing on stems while the walnut root fungus is adapted for growing on roots. The pineapple odour, which is strong when these fungi are grown on autoclaved walnut material, is almost absent when they are grown on fresh material.

The macroscopic appearance of plate cultures on carrot agar, potato dextrose agar, and malt agar has been recorded. The maximum and minimum diameter of each culture was measured every second day, and the mean between these two measurements was recorded for each plate. These observations show that the four strains of *Chalaropsis* tend to form a series, at one end of which is the walnut graft strain, which usually forms light-coloured cultures, regular in outline, while the carrot strain, at the other end of the series, produces typically fringed growth, which is dark green or nearly black. The other two strains are intermediate, the peach strain being somewhat similar in external appearance to the walnut graft strain and the fungus isolated from the walnut root approaching the carrot strain (Fig. 7).

IV. PHYSIOLOGY.

A. *Temperature Relations.*

The type of growth obtained at different temperatures was studied for each of the four strains of *Chalaropsis*. Plates of 20 per cent. carrot agar were inoculated with a suspension of spores and incubated at various temperatures from 0° C. to 35° C. As these fungi did not grow at 0° C. or at 35° C., the experiment was repeated using the following range of temperatures: 5° C., 12° C., 18° C., 25° C., 30° C., 33° C. At intervals of two days the growth on each plate was measured, the smallest and largest diameters being recorded in every case.

All the strains produced very little growth at 5° C., this increased slightly at 12° C., reaching a maximum between 18° C. and 30° C.; at 33° C. there was again very little growth. It is interesting to note that the peach strain grew equally well at 30° C. as at 25° C., whereas in all the other strains growth was considerably less spreading at 30° C. than at 25° C. This observation suggests that the peach strain is adapted to its host plant, which normally grows in hot-houses or warm climates. The carrot strain grew most readily at 18° C. This again may be an adaptation to the environment of its host, which usually grows out-of-doors in temperate climates. The optimum temperature for the walnut root strain was 25° C., and the walnut graft strain grew equally well at 18° C. and 25° C. All the strains produced abundant endospores after being incubated for two days at 30° C., 25° C. or 18° C.

The carrot strain became dark in colour most readily, while the walnut graft strain remained white throughout the whole range of temperatures, only becoming yellowish at 33° C. as the cultures dried out. The walnut root and peach strains were intermediate in the series, the walnut root cultures usually first assuming a yellow colour.

During the month that these plates were observed, certain well-marked changes took place in the outline of the cultures. The walnut graft strain remained regular throughout the whole range of temperatures at which it was grown. The carrot strain, in contrast, developed a lobed outline even when grown at a temperature as low as 12° C. The walnut root and peach strains were again intermediate, as they developed lobed and fringed cultures at 18° C. and 30° C., and at 18° C. and 33° C. respectively.

A characteristic type of growth was noticed in the peach strain. Dark greyish-green sectors invariably occurred in cultures of this strain when growing at 12° C. or at 18° C. The dark colour in these sectors was due to mycelium in which the cell-sap was olive-green.

B. *pH Relations.*

The type of growth obtained at various degrees of acidity was studied for the four strains of *Chalaropsis*. By using phosphoric acid and sodium hydroxide, carrot agar was adjusted so that after sterilization the reaction was pH 3·5, 5·2, 6·7, 8·0, 9·0.

After inoculation, the plates were incubated at 25° C. and the growth on each plate was measured every second day. None of the strains produced much growth when the medium was acidified to pH 3·5 but all the strains produced spreading growth at the remaining pH values, and there was little variation between them. As the pH value of the medium was increased, endospores were produced less abundantly.

Cultures of all the four strains of *Chalaropsis* when grown on carrot agar adjusted to pH 5·2, 6·7 or 8·0, developed numerous, characteristic growth lines radiating from the centre. Where endospore production was vigorous, as in cultures growing at pH 5·2, the spores occurred chiefly in radiating lines, but where the cultures consisted largely of mycelial growth, the mycelium was comparatively dense in these radiating lines.

It was generally noticed that in any one strain, an increase in the pH value of the medium resulted in cultures of a deeper colour, until pH 6·7 was reached ; above this value, however, the colour decreased as the medium became more alkaline. As noticed in previous experiments, the colour produced in cultures of the carrot fungus was always deeper than that produced in comparable cultures of other strains.

With increased pH, and with consequent decreased production of endospores, drying-out of the cultures took place more quickly. Finally, on medium adjusted to pH 9·0 the mycelium was so thin that it was barely visible to the naked eye. As cultures on this alkaline medium were so abnormal, it was found impossible to state definitely when drying-out commenced in this case.

C. *Enzymes.*

Only qualitative tests were made for the presence of diastase, oxidase, cytase, pectase and emulsin. Four flasks of sterile carrot extract were inoculated respectively with the four strains and incubated at 25° C. for one month. To test for intracellular enzymes, the growth in each flask was treated by a method which was similar to that described by Dox (5), so that finally the mycelium was obtained in the form of a coarse, dry powder which was stored in a tightly-corked specimen tube and kept in the dark.

After removal of the mycelial discs from the original flasks of carrot extract, this extract was filtered and the clear liquid obtained in each case was tested in addition to the powdered mycelium, for the presence of enzymes.

It was found that diastase, oxidase and cytase were present in these fungi, while pectase and emulsin were not detected. Cytase does not diffuse from the fungi into the culture media, but diastase is found to some extent in the culture media. In contrast with these two enzymes, a more pronounced oxidase reaction was given by the used culture media than by the actual suspensions of enzyme powders.

V. THE FUNGUS IN NATURALLY-INFECTED TISSUES.

The appearance of *Chalaropsis* in naturally-infected tissues has only been studied on young walnut trees. The fungus always enters through a wound or at a cut surface and seems unable to penetrate the uninjured epidermis.

In order to study the microscopic appearance of the fungus in the host, transverse and longitudinal sections were cut through naturally-infected tissue. These were stained in cotton blue dissolved in lacto-phenol, the surplus stain being washed out in lactic acid, leaving the mycelium and cell contents of the host stained blue. The distribution of the fungus was made clear by this method.

A. *Walnuts at the time of grafting.*

Walnuts are grafted under glass in February and March. After grafting, the pots are plunged into coconut fibre in closed frames, with a bottom heat of about 70° F. The atmosphere is kept very moist and conditions are ideal for the fungus to develop. After three or four days, unless certain precautions are taken, white powdery endospores appear on naturally-infected grafts at the junction between stock and scion, and about ten days after grafting, macrospores develop on the cut surfaces of the stocks and scions.

1. *Rootstock.* When grafting, two parallel, longitudinal cuts are made through the stock, so that one of these cuts splits it down the centre, while the other, made externally to it, passes through wood and cortex but not through the pith. Thus half the stock is left intact. Several of these half-stocks, taken from naturally-infected material, were used for sectioning. Transverse sections showed macrospores and endospores being produced on normal conidiophores all along the cut surface, from cortex to pith. Owing to the lack of light and air, macrospores were more abundant than endospores. The latter were only found in profusion at the exposed edges of the longitudinal cut. On diseased stocks the bark was frequently broken, exposing spores of both types borne on normal conidiophores. Cells of the cortex were usually brown at the cut surface, and isolated strands of sparsely-septate and sparsely-branched mycelium were noticed throughout the bark and cortex. In addition to these isolated strands of mycelium, groups of cells in the cortex contained numerous, coiled strands of mycelium (Fig. 5). These cells, which contained masses of hyphae, were frequently dark brown.

As walnut scions are grafted on the upper part of the tap-root, the wood vessels and fibres of the stock are not much thickened at the point of union. Most of the mycelium in this area was found in cells of the medullary rays, running in a direction parallel to the rays. Strands of hyphae were also seen passing across the fibres and vessels. At the cut surface, cells of the medullary rays were often brown. Spores were produced actually within the pith cells (Fig. 6), and septate, branched mycelium of varying diameters traversed the pith in all directions.

2. *Scion.* As in the stock, the fungus was studied in sections taken from approximately half the scion, which, after grafting, is left intact at the point of union. The general distribution of the fungus was similar to that in the rootstock. In transverse sections both forms of spore were seen all along the cut surface, but macrospores were much more abundant than endospores. The proportion of cortex to wood was small in the scions, and there was relatively less mycelium in the cortex of the scion than in the cortex of the rootstock, cells packed with mycelium being rarely seen.

In transverse sections of the wood, thin, septate strands of mycelium tended to cross the thick-walled, wood-fibre cells and to pass along the medullary ray cells, parallel to the rays, as noticed in sections of the rootstock. Mycelium was also abundant around and within the vessels, and macrospores were produced actually in the vessels. The pith in a walnut scion is chambered, and cells are only found at its periphery. These cells contained branching mycelium and conidiophores bearing spores.

B. *Roots.*

When walnut seedlings are lifted from the nursery, preparatory to grafting, the long tap-root almost invariably has to be trimmed before the plant is put into a pot. The cut surface which is left forms a point at which *Chalaropsis* can enter the host (Fig. 7). In this case *Chalaropsis* spores, presumably from the soil or from the walnut root, must have entered the stock before a pad of callus tissue had been formed over the cut surface. Meanwhile, the stock was grafted, as it appeared healthy above ground. A union took place and the scion made several inches of growth before the plant began to wilt. On examination it was found that although the union was perfect the stock had died from the base of the root upwards. Sections of similar roots, in which infection was less advanced, were cut and investigated.

At the cut surface it was impossible to section the root, as the cortex and central cylinder consisted of a black mass of disintegrating cells and macrospores. Further up the root the central cylinder was still disintegrated, but the cortex, although black, remained intact. In this area, the cortex showed extensive groups of infected cells containing macrospores and knots of hyphae as noticed in the cortex of stocks infected at the point of union. In infected areas, the cells contained no starch, but these cells often adjoined patches of normal cortical cells packed with starch grains. Between infected and healthy tissue a meristematic layer was usually noticed; such cells are presumably formed in an attempt to isolate the diseased areas.

In making sections of the root from the base upwards, the cortex was found to be more and more normal, until the blackened areas of cells were no longer present. The central cylinder in this part of the root was intact, but remained black. This black colour was due to the presence of macrospores and to the dark colour of the infected cells. This dark colour persisted for the greatest length in the medullary rays. Mycelium and macrospores were most abundant in the xylem parenchyma and in the medullary rays, and were seen to a less extent in the vessels. Higher up the root, where the central cylinder was more normal, starch grains were present in the medullary rays and in the xylem parenchyma, infected cells without starch being restricted to small areas, and these finally were absent. In every root examined, the fungus had travelled farther in the central cylinder than in the cortex, and the medullary ray cells were attacked more readily than any other type of cell.

C. *Rooted layers.*

Rootstocks for walnut grafting are also being raised vegetatively by the layering method. In this process, rooted layers are cut from the stools in the autumn and planted in nursery rows. Infection by *Chalaropsis* spores frequently takes place at the cut surface which is exposed on the layer when it is removed from the stool. On examining an infected layer, a powdery mass of black spores is seen at the cut surface, but the cells of the host are not broken down as in infected roots. This difference between roots and layers is presumably due to the fact that the structure of a rooted layer, which is that of a stem, is more lignified, and therefore less liable to disintegration by fungi, than a one-year-old tap-root. In a rooted layer, the fungus travels farther in the cortex than in the highly lignified central cylinder. As mentioned above, the reverse is true in a tap-root. Here, the wood is much softer, and, owing to the greater length and more parallel arrangement of the cells, the fungus can travel more readily in the central cylinder than in the cortex. The distribution of the fungus in a layer is similar to that in a scion infected at the time of grafting.

VI. INOCULATION EXPERIMENTS TO TEST THE PARASITISM OF THE *Chalaropsis* STRAINS.*

A. *Walnuts.*

1. *Grafts.* In March of 1932, six seedling rootstocks of *Juglans nigra* were grafted under glass with well-ripened scion wood of the variety Franquette. Sterile knives were used and the stocks were also sterilized by painting with

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* As these inoculation experiments were carried out before the strain of *Chalaropsis* isolated from peach seedlings had been received, the strains tested in this section were restricted to those isolated from walnut grafts, walnut roots and carrots.

1 per cent. formalin* before grafting. At the time of grafting, growth from a culture of the walnut graft strain of *Chalaropsis* on carrot agar was inserted between the stock and the scion. As a control, six similar rootstocks were grafted under sterile conditions with the same variety. The twelve grafted plants were plunged into coconut fibre in the same closed frame, and sprayed frequently.

At the end of four days, a powdery white mass of endospores was seen on three of the inoculated plants at the cracks in the stocks and scions which marked the upper and lower extremities of the unions. Within a fortnight, all the inoculated scions were discoloured just above the union, while the controls remained healthy. Later, the discoloured areas on the inoculated scions spread upwards and several of the buds became rotten and fell off. Meanwhile, pads of callus tissue began to break through the grafting-wax at the unions of the control plants, and the buds grew large and green. At the end of one month the control plants had put out new shoots, with the exception of one plant in which the buds had swollen preparatory to growing out. The inoculated scions were dead at this time, and saprophytes were beginning to appear on them.

This inoculation experiment was repeated under similar conditions in March 1933, and changes comparable to those noted in the previous year took place in the inoculated and control plants. This series of plants was photographed two months after the grafting was carried out (Figs. 9, 10). In Fig. 9, saprophytic fungi can be seen on the inoculated scions, and light-coloured patches, which consist of masses of *Chalaropsis* endospores, are obvious on the junctions between the stocks and scions. Fig. 10 shows that each control plant has made good growth, most of the shoots being from four to six inches long, and bearing three or four strong leaves. In several cases pads of callus can just be detected at the unions between the stocks and scions, and the original strings which were used at the time of grafting, have been broken by the callus tissue, so that each graft has had to be re-tied with thick string.

On untying the dead grafts, macrospores, looking like soot to the naked eye, and proving to be morphologically typical of *Chalaropsis*, were found at the unions. Sections were cut through inoculated stocks and scions, and the distribution of the mycelium was found to be similar to that described for naturally-infected grafted walnuts.

2. *Roots*. Twelve one-year-old seedling rootstocks of *Juglans nigra* were lifted from the open ground in November 1931 and bedded in. In February 1932, the tap-roots were trimmed preparatory to planting in pots, and sterilized by dipping in 1 per cent. formalin. When dry, a deep, transverse cut was made just above the base of each root, and growth from a culture of the walnut root

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* See Section on Control Measures.

strain of *Chalaropsis*, growing on carrot agar, was inserted in the cuts on six of the seedlings while the others were left as controls. Each seedling was then potted and left in a cool greenhouse until the following autumn.

During the summer no difference could be detected in the vigour of the control and inoculated plants, some plants in each batch making poor growth, while the rest grew normally. When the seedlings were uprooted in the autumn, the typical disintegration of the base of the tap-root associated with *Chalaropsis* infection, was found to some extent on each of the inoculated plants, while the control plants showed normal pads of callus tissue. When this experiment was repeated in 1933, typical infections were again produced on the inoculated tap-roots, and four of the controls had healed normally. The other two control plants, however, showed a slight blackening of the cut surface, which, on microscopic examination, was seen to be associated with *Chalaropsis* macrospores. This experiment indicates that root infection can take place from the soil, although it takes place more readily from spores in association with the tap-root when it is potted.

3. *Shoots.* During June 1933, spore suspensions of pure cultures of the strains of *Chalaropsis* isolated from a walnut graft, walnut root and carrot were inoculated into T-shaped cuts on walnut shoots. Young grafted walnut trees growing outside and in the greenhouse were used for these inoculations, and the parasitism of each fungus was tested on shoots of the current year and of the previous year.

The inoculations were examined twice a week for a month, and after this time at less frequent intervals. No difference was detected between inoculations in the greenhouse and outside, and similar results were produced by all three strains. At the end of three days a slight blackening of the edges of the cuts on young shoots was observed, and a few endospores were present beneath the bark. The inoculations on old wood and all the controls were unchanged at this time. Within a fortnight, macrospores and endospores were detected beneath the bark on all inoculated cuts, and the bark had blackened for about 0·5 cm. around each cut, while the wood, which had been exposed by the shrinkage of the bark, was brown. Spores were absent on the control lesions, which became only very slightly discoloured at the edges of the cut. A month after the inoculations were made, endospores were less obvious and the blackened areas around the lesions were not extending, while the control cuts were callusing normally on old and young wood. During the latter part of July and in August, callus tissue was also formed on the inoculated cuts, and, although macrospores and endospores remained on the exposed wood and on the callus, the fungus made no further progress. Transverse sections of inoculated shoots showed a discoloration of all exposed tissue. In the medullary rays, this discoloration spread through a depth of several cells. Spores were borne in the crevices between the bark and the wood, and in the pith, while scanty strands of mycelium were found in superficial cells.

Another series of experiments was carried out in which the same three strains of *Chalaropsis* were inoculated into walnut shoots, the spore suspension being inserted just beneath the surface in each case. The object of these experiments was to determine whether the fungus could penetrate the wood if this was uninjured. Both types of spores were produced beneath the epidermis of inoculated cuts on old and young wood, but there was very little discoloration, and control and inoculated cuts had callused over within six weeks. On cutting sections through these lesions, a few strands of mycelium were found passing between the cortical sclerenchyma groups adjoining the point of inoculation. Mycelium was then found in the phloem, and finally it was seen in a few cells of the xylem.

In order to determine whether *Chalaropsis* would cause more damage to walnut shoots when the trees were dormant, trees out-of-doors and in the greenhouse were inoculated in November by spore suspensions placed in the usual T-shaped cuts. The subsequent blackening of the lesions and production of spores took place more slowly than when similar inoculations were carried out in the summer, and although callus formation did not take place until the following spring, there was no increase in the damage caused by the fungus.

These experiments indicate that *Chalaropsis* does very little damage when inoculated into shoots of established walnut trees. Although lesions are formed, the production of a pad of callus tissue prevents the fungus from girdling the shoot.

B. *Carrots.*

As one of the strains of *Chalaropsis* was originally isolated from diseased carrots, the parasitism of this strain and of the walnut strains was tested on this host. In the spring of 1933, seeds of dwarf carrots were sown under glass; later they were potted singly and kept in the greenhouse. Early in June the carrots were inoculated, a drop of spore suspension being inserted in a cut made in the upper part of the carrot root, which was just visible above the level of the soil.

At the end of three days, a powdery mass of endospores had been produced at the cut surfaces of all the inoculated carrots. The layer of endospores was most definite in the inoculations where the strain of *Chalaropsis* obtained from carrots had been used. Within a week, a grey colour indicated the presence of macrospores on the lesions, the darkest colour being produced by the carrot strain. At this time it was noticed that where the strain from the carrots had been used, the lesions had become larger and deeper, while cuts inoculated with the walnut strains had not increased in size. At the end of three weeks, the cuts on the control carrots had callused over, and those on carrots inoculated with the graft strain had also begun to heal. Lesions produced by the walnut root strain had increased in size, the carrots being discoloured for about 0·5 mm. around the actual lesion. The most severe attacks were due to the carrot strain. Here, the original outline of the cut had disappeared, and one side of the upper part of the carrot was disintegrated and covered with spores. The carrots were discoloured for about 1·0 mm. around the infected areas.

As the carrot strain of *Chalaropsis* was able to enter a carrot through a wound and produce a severe infection, an experiment was carried out to determine whether the fungus could penetrate the epidermis in the absence of a wound. Inoculated and control carrots were kept moist with sterile cotton wool, and were examined at frequent intervals. At the end of a week, a slight blackening was noticed on the surface of inoculated carrots, but the infections never progressed any further.

C. *Lupins.*

As *Chalaropsis* was originally described as a parasite of lupins in Italy, inoculations were carried out to determine whether these strains would attack lupins. Seedlings were potted up singly and inoculated at the base of the stem with spore suspensions obtained from cultures of the walnut graft, walnut root and carrot strains of *Chalaropsis*.

Within ten days, definite lesions had been produced on each of the inoculated lupins, and both types of spore were present at the cut surfaces, the dark grey colour associated with macrospores being more evident on lesions produced by the walnut root and carrot strains than in those produced by the walnut graft strain. At the end of three weeks, cuts on the control lupins had callused over, while those on the inoculated plants, although black in colour, had progressed no further. All the plants appeared to be growing equally vigorously. Two months after inoculation, all the cuts had callused and the plants were in bud or full flower. This experiment indicates that, under normal conditions, these strains of *Chalaropsis* are only very weakly parasitic on lupins, although, when crowded and grown in a moist atmosphere, it is possible that inoculated seedlings would suffer greater injury.

VII. CONTROL MEASURES.

A. *Experimental.*

Experiments with hanging-drop cultures were carried out in the laboratory, in order to discover solutions which were toxic to *Chalaropsis*. As the fungus is most resistant at the macrospore stage, these spores, obtained from a naturally-infected seedling stock, were used throughout the tests. The following method was adopted. A dense suspension of macrospores was prepared in sterile water, and a drop of this transferred to each of a number of sterile coverslips. When the water had evaporated, a drop of one of the solutions to be tested was applied to the spores on each coverslip, which was then set up as a hanging-drop culture. Control cultures of spores in sterile water only were set up in the same manner.

After twenty-four hours the drops were washed off the coverslips with sterile water, leaving the spores behind, and, in order to give the spores favourable conditions for germination after treatment, the test solutions were replaced by drops of carrot extract. Spores which did not germinate after a fortnight in carrot extract had presumably been killed by the treatment.

Of four solutions tested, the lowest concentrations required to inhibit germination under these conditions were:

Mercuric Chloride	0·0075 per cent.
Copper Sulphate	2·5 ,, ,,
Cheshunt Compound	3·0 ,, ,,
Formaldehyde	0·5 ,, ,,

As formaldehyde has proved to be so toxic to *Chalaropsis* macrospores, and since it can be conveniently manipulated, further tests of its use for the control of the fungus were carried out. Sixty c.c. of commercial formalin was vaporized with a spirit lamp in a small fumigation chamber of 176 cu.ft. capacity. Macrospores from a naturally-infected walnut graft were placed on glass slides which were exposed on the floor of the fumigation chamber. Some spores were moistened with a drop of water, while others were left dry. Control slides with comparable spores were left in a cool greenhouse. After fumigation had proceeded for twenty-four hours, spores from each slide were transferred to drops of carrot extract on coverslips, and set up as hanging-drop cultures at room temperature. At the end of eighteen hours, all samples from the control slides showed a high percentage of germination, while spores from slides exposed to formaldehyde remained unchanged. Even at the end of a fortnight these spores had not germinated, thus showing that formaldehyde gas at this concentration is toxic to *Chalaropsis* spores.

B. *Application.*

As a result of these laboratory experiments, formalin has been generally used to control *Chalaropsis* infection at East Malling, as it is effective in low concentrations, even when macrospores are exposed to ideal conditions for germination after treatment; it is also cheap and can be used without injury to the walnuts. Moreover, it has one great advantage over the rest of the substances used, in that it is volatile and so can readily be eliminated when sterilization has been effected. In practice, 1 part of commercial formalin (40 per cent. formaldehyde) with 39 parts of water is used to ensure good control. It is now used each spring, before walnut grafting commences. The greenhouse is cleaned out and then sprayed with formalin, care being taken to penetrate all crevices in which spores might lodge. Before the walnut stock is cut, preparatory to inserting the scion, the point of union is painted with formalin. As a result of these precautions the fungus has almost disappeared, and 93 per cent. success was obtained with good, well-ripened walnut scions in 1933, compared with about 5 per cent. in 1930, when the fungus was first observed.

Formalin is also used to sterilize the cut surfaces which are formed when rooted layers are removed from the parent stool before planting. Formalin is used in the same way on the cut surfaces of seedling stocks where these have been trimmed preparatory to potting up. Inoculation experiments have shown that a complete control of *Chalaropsis* is not obtained by this method of sterilizing the cut surfaces of seedling stocks with formalin ; it is presumed that these results are due to the presence of *Chalaropsis* spores in the soil.

VIII. SYSTEMATIC POSITION OF THE FUNGI STUDIED.

A fungus corresponding morphologically to the one isolated from walnut has been recorded on one previous occasion only. In 1916 Peyronel described a parasite of lupins in Italy (8), which resembled *Thielaviopsis basicola,* the fungus which causes a root rot of tobacco, but was quite distinct from it, in that the macrospores were borne singly and terminally, instead of in chains. He therefore decided to erect a new genus and species, with the name *Chalaropsis thielavioides*.

The four fungous strains described in this paper bear a general resemblance to *Chalaropsis thielavioides*. Peyronel records the presence of two spore forms, the macrospores borne singly and terminally on short conidiophores often in clusters, while the microspores are always endogenous and borne in chains. As microspores are formed in abundance under moist, humid conditions, Peyronel suggests that their chief function is to spread the fungus rapidly, whereas the macrospores enable it to survive unfavourable conditions. Both conidiophores and vegetative mycelium are hyaline, but darken with age and appear olive-green when seen in mass.

Unless conditions are excessively humid, lupins which are not subject to secondary infections, continue to grow and flower normally, only the weaker plants being killed by *Chalaropsis*. Similarly, *Chalaropsis* has been observed to attack walnuts only through wounds, and it only assumes importance during walnut grafting when the plants are subjected to abnormal conditions of temperature and humidity, and large cut surfaces are available through which the fungus can enter.

Having entered a lupin stem at a superficial lesion, the fungus develops in the cortex, forming dense knots of hyphae which completely fill the cell cavities ; a similar appearance has been noticed in the cortex of a walnut shoot attacked by *Chalaropsis*. On lupins, *C. thielavioides* is unable to penetrate the vascular bundles, but usually develops exclusively in the cortex and phloem, giving rise to abundant macrospores. The deep wounds which are made in walnut shoots and roots during grafting, allow the fungus direct access to the xylem and cannot therefore be compared to the superficial wounds through which lupins become naturally infected. It is found, however, that when walnut shoots are inoculated artificially, the fungus is able to penetrate the cambium layer and enter the wood. This difference in the distribution of the fungus in the host plants is the only one which has been consistently noted between *Chalaropsis* on the walnut in England and that on the lupin in Italy.

Many points of similarity have also been noticed in *Chalaropsis* and species of *Thielaviopsis*. In both genera there are two conidial forms, the microspores being endogenous. The chief difference between these genera is that macrospores are borne singly and terminally in *Chalaropsis*, while in *Thielaviopsis* they always occur in chains and may be endogenous like the microspores. The tendency to chain-formation noted in the strain of *Chalaropsis* isolated from peach seedlings may have some significance in this connection. Another difference between these genera is that endoconidia in *Thielaviopsis* do not always remain hyaline, but may develop a dark coloration as in *T. paradoxa*, so that when mature they can hardly be distinguished from the macrospores. The olive-green coloration which develops with age in the mycelium of *T. paradoxa* is comparable to the colour changes which take place in *Chalaropsis*. Another point of resemblance between *T. paradoxa* and *Chalaropsis* is the distinct pineapple odour which is always present in cultures which are growing freely. In his paper, Peyronel makes no mention of this odour for the lupin fungus, but in a letter received from him in 1931 he wrote: "Cultures of *Chalaropsis* on carrots and other usual cultural media produce, like those of *Thielaviopsis*, a smell resembling that of ripe pineapples."

The method of production of endospores in *Chalaropsis* isolated from walnut appears to be different from that in *T. basicola*. In the latter, as described by Brierley, after the production of the first endospore, which is cut off by a wall from the rest of the contents of the endoconidiophore, the conidiophore is ruptured, the apex being broken off like a lid with an irregular edge, exposing the first spore (3). In *Chalaropsis* such a lid has not been observed, but a definite split occurs at the apex of the conidiophore, through which the endospores are forced by vacuolation, which takes place at the base of the endoconidial cell.

As the genera *Chalaropsis* and *Thielaviopsis* are so similar, both in the morphology of their conidial stages, and in their mode of parasitism, it seems natural to class them together systematically, although the perfect stage of *Chalaropsis* has not yet been obtained.

In those species of *Thielaviopsis* where the perfect stage has been found, it has proved to be a *Ceratostomella* and the perithecia terminate in a beak through which the ascospores are discharged. Originally, the perithecia found on sweet potato were thought to be pycnidia, and the fungus was called *Sphaeronema fimbriata* or *Ceratocystis fimbriata,* but when these fructifications were found to be perithecia the fungus was re-named *Ceratostomella fimbriata.* Similarly, the fungus which causes Black Rot of sugar cane was first named *Sphaeronema adiposum* on account of fructifications which occurred at the cut ends of seed-cane. Later, the fructifications were recognized as perithecia of a *Ceratostomella,* and therefore the name of this fungus was changed to *Ceratostomella adiposum. Thielaviopsis paradoxa* is a heterothallic fungus, as shown by Dade (4); hence the ascigerous stage, which is known as *Ceratostomella paradoxa,* is only produced in the presence of two complementary strains. The perfect stage of *Thielaviopsis basicola* has not yet been found.

About the year 1878, Berkeley received some walnut shells from Dr. Buchanan White of Perth, in Scotland. These shells had black marks on them due to a fungus which produced septate endoconidiophores and cylindrical, hyaline endoconidia. Berkeley identified this fungus as *Cylindrosporium longipes* Preuss. (1). Later he revised this decision, as the ampulliform conidiophores, characteristic of the genus *Cylindrosporium,* were absent in this fungus. He therefore renamed it *Chalara longipes* (2). The original walnut shells are now in the Herbarium at Kew. A microscopic slide of the fungus present on them, prepared by Berkeley, was lent to the writer by Mr. Mason, of the Imperial Mycological Institute. This slide showed conidiophores and endoconidia similar to those of the walnut graft fungus, so a visit was paid to the Herbarium to make a more detailed examination of the material. On this occasion ovoid, brown macrospores were found in addition to the endospores, and the dimensions of these were similar to those found on walnut grafts. In view of this, it is now considered that the endospores and macrospores on the walnut shells in the Kew Herbarium are two spore forms of the same fungus which has recently been relegated to the genus *Chalaropsis.* It is probable, therefore, that the fungus described in this paper as the cause of failure in walnut grafting was present in Britain as long ago as 1878.

In 1902, McAlpine (7) published an account of: "Fungus Diseases of Stone-fruit trees in Australia and their treatment." In reviewing the root diseases, he mentions two new species which are of interest in this discussion. The first occurred on the bark of apricot roots and bore elongated, cylindrical, hyaline or smoky-brown spores. These spores are not described as endogenous, but they are figured as emerging from a cylindrical sheath. McAlpine named this fungus *Cylindrium intermixtum.* The genus *Cylindrium,* as generally understood, includes fungi bearing elongated, hyaline spores, which are not endogenous, while fungi with spores which are endogenous, such as those figured by McAlpine, would be placed in the genus *Chalara*.

The second fungus was found associated with the *Cylindrium*, also on apricot roots, chiefly between the bark and the wood. McAlpine describes the conidia as "terminal, generally borne at the end of short branches, globose to sub-globose or irregular, brown, smooth, thick-walled, average 15-19μ diameter. . . . The conidia are borne in large, irregular clusters like a bunch of grapes". He adds that "there is every indication of this species being parasitic, as it was found on living tissue both on the bark and between the bark and the wood". McAlpine named this fungus *Coniosporium radicicolum*.

In view of Peyronel's work on *Chalaropsis thielavioides*, it seems very probable that McAlpine was dealing with a fungus which was similar to, if not identical with that described by Peyronel as a parasite of lupins in Italy. In support of this theory, it was noticed that in one of McAlpine's figures, a tube, similar to an empty endoconidiophore of *Chalaropsis*, is shown on the same hypha immediately below one of the brown, sphaeroidal spores, which were attributed to *Coniosporium radicicolum*. McAlpine, however, did not recognize any genetic connection between the two forms, and described them as distinct fungi. His description (though brief) and figures conform closely with those of the walnut fungus.

Of the fungi described in this paper, the three strains obtained from walnut grafts, walnut roots and peach seedlings respectively are indistinguishable morphologically ; although they show some cultural difference, these are not considered to have any special significance. These fungi are therefore thought to be different strains of one and the same species and they conform with Peyronel's description of *Chalaropsis thielavioides*. Moreover, a culture of the graft strain was sent to Professor Peyronel in 1931 for his opinion as to its relation with the lupin fungus. He replied: "It is really the very fungus I studied on *Lupinus*: both morphological characters and the peculiar flavour [odour] are identical, so I think you can without hesitation consider it as being *Chalaropsis thielavioides* ; at the most may it be distinguished as a biological form."

Until the perfect stage of this fungus has been found, it is proposed therefore to accept Peyronel's species *Chalaropsis thielavioides* as the cause, not only of a rot of lupins in Italy, but also of a disease of young walnut trees following grafting in England.

The carrot strain shows some distinct differences from the other three strains in morphological and cultural characters. The fact that it is definitely more parasitic on carrots than either of the walnut strains suggests that it is biologically specialized to attack this host, or even that it may be a distinct species. The morphological and cultural characteristics shown by the strain support this view. Until they have been studied more closely, however, it seems preferable to include the carrot strain, with the other three described in this paper, under the name *Chalaropsis thielavioides*.

IX. DISTRIBUTION OF *Chalaropsis thielavioides*.

Chalaropsis thielavioides has thus been recorded by Peyronel in Italy as a parasite of lupins, and in England as attacking young walnut trees. The discovery of typical endospores and macrospores on walnut shells which were received by Berkeley from Scotland indicate that the fungus also occurs there.

Peach trees received in Palestine from France had *Chalaropsis thielavioides* on them, so it may be assumed that this fungus is also present in France.

McAlpine's description and figures of the two forms he found associated on roots of apricot, when compared with Peyronel's work on *Chalaropsis thielavioides*, suggest that the same fungus is also present in Australia.

SUMMARY.

The constant appearance of a fungus with two spore forms, on young walnut trees that failed to grow after grafting, suggested that this fungus was the cause of the failure.

The organism was isolated in pure culture and its relation with the disease investigated. The genetic connection between the two spore forms was established, and the fungus was found to be a species of *Chalaropsis*.

Three other strains of *Chalaropsis* and a species of *Thielaviopsis* have also been grown in culture ; their morphological characters and cultural relations have been compared.

The appearance of naturally-infected walnut tissues and the distribution of the fungus in them are described.

Inoculations on walnut grafts gave positive results and definite evidence was obtained that the fungus is the cause of the disease. The carrot strain was the most virulent as a parasite of carrots.

Laboratory control tests showed the formalin at low concentrations is toxic to *Chalaropsis*. It is now generally used for controlling the disease.

The resemblance between the strains of *Chalaropsis* described in this paper and Peyronel's lupin fungus appears to justify the name *Chalaropsis thielavioides* for all these forms. The probable distribution of the fungus is discussed.

ACKNOWLEDGMENTS.

The writer wishes to thank Dr. H. Wormald for his help and advice throughout this investigation, and Mr. A. W. Witt for assistance with the grafting

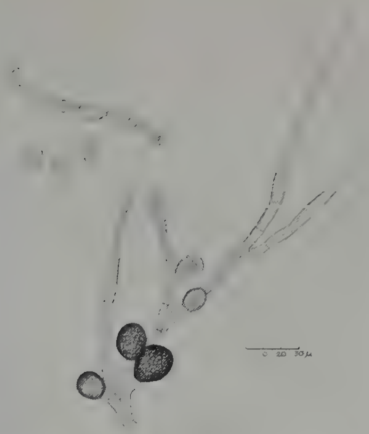

FIG. 1.

Mycelium of walnut root strain showing production of macrospores and endospores, and swollen endospores. Growth taken from culture on carrot agar.

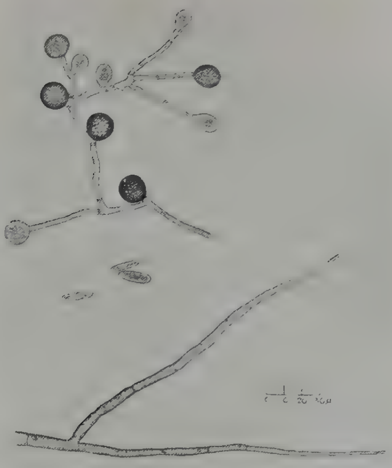

FIG. 2.

Mycelium of carrot strain showing production of macrospores and endospores, and swollen endospores. Growth taken from culture on carrot agar.

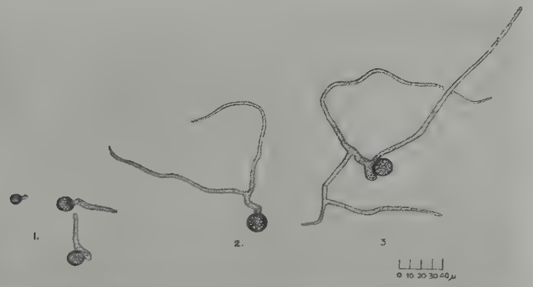

FIG. 3.

Macrospores of the carrot strain germinating in carrot extract. At the end of (1) 24 hours; (2) 48 hours; (3) 72 hours.

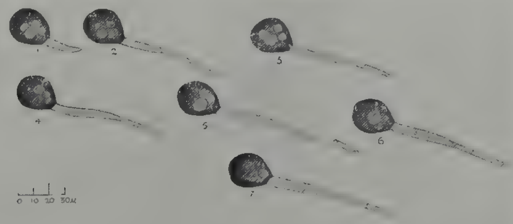

FIG. 4.

Stages in germination of a macrospore from a naturally-infected walnut seedling stock. At the end of (1) 15 hours; (2) 18 hours; (3) 18 hours 30 minutes; (4) 19 hours 6 minutes; (5) 19 hours 11 minutes; (6) 19 hours 16 minutes; (7) 19 hours 52 minutes.

PLATE II.

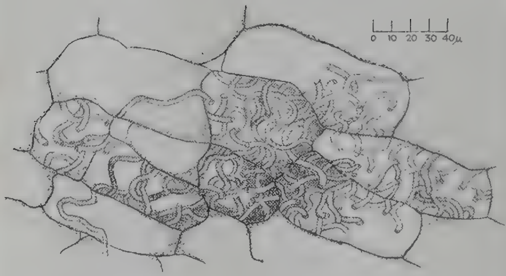

Fig. 5.

Transverse section showing a group of infected cells in the cortex of a walnut seedling stock.

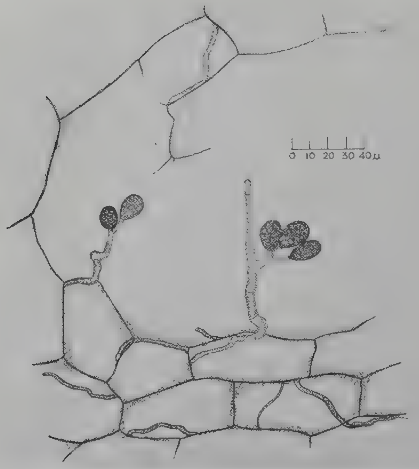

Fig. 6.

Transverse section showing macrospores and endospores in the pith of a walnut seedling stock.

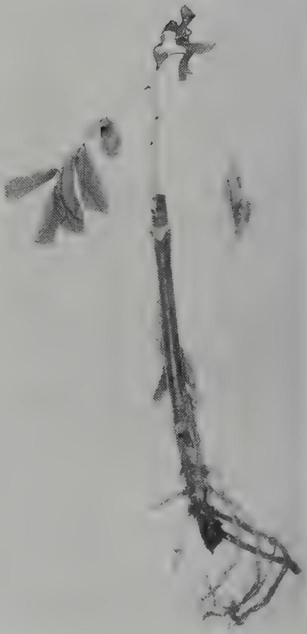

Fig. 7.

A grafted walnut which has died as a result of infection of the tap-root by *Chalaropsis thielavioides.*

PLATE III.

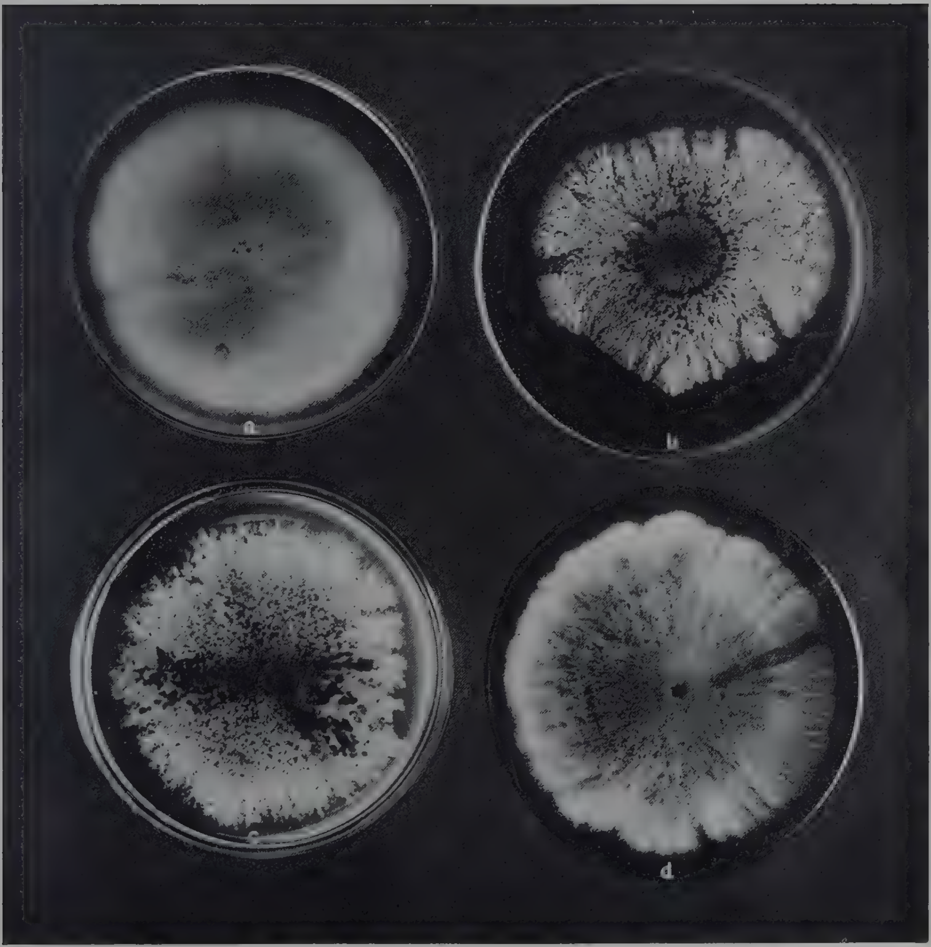

FIG. 8.

18-day-old cultures on potato dextrose agar, (*a*) Walnut graft strain ; (*b*) Walnut root strain ; (*c*) Carrot strain ; (*d*) Peach strain.

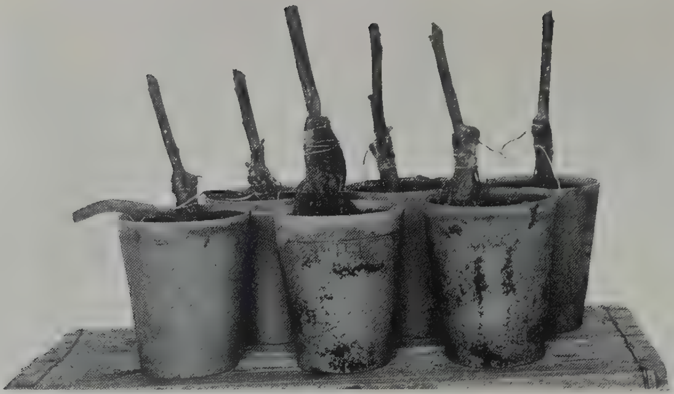

FIG. 9.

Grafted walnuts photographed two months after inoculating with walnut graft strain of *Chalaropsis thielavioides*.

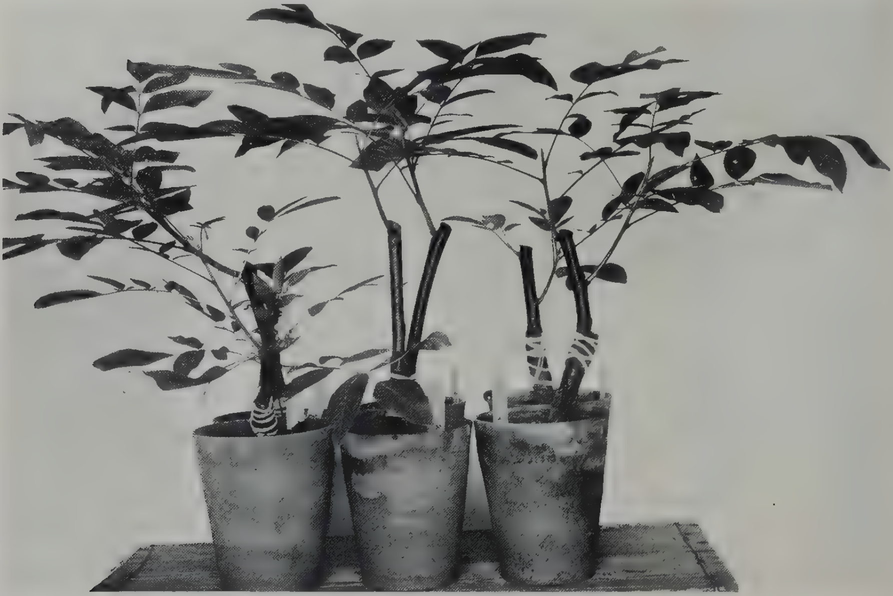

FIG. 10.

Control grafted walnuts photographed two months after grafting.

experiments. She is also indebted to Miss E. M. Wakefield and to Mr. E. W. Mason for information concerning the systematic position of *Chalaropsis*. Thanks are also due to Mr. E. W. Mason and to Dr. A. Smith for supplying cultures of *Chalaropsis*, and to Professor Peyronel, of R. Istituto Superiore Agrario, Firenze, Italy, for examining and reporting on a culture of the fungus isolated from walnut.

REFERENCES.

(1) *Berkeley, M. J.* Ann. Mag. Nat. Hist. (1878), 5th series, **1**, 26.

(2) ———. Ann. Mag. Nat. Hist. (1881), 5th series, **7**, 130.

(3) *Brierley, W. B.* The "Endoconidia" of *Thielavia basicola* Zopf. Ann. Bot. (1915), **29**, 483-93.

(4) *Dade, H. A.* *Ceratostomella paradoxa.* The perfect stage of *Thielaviopsis paradoxa.* Brit. Mycol. Soc. Trans. (1924), **13**, 189.

(5) *Dox, A. W.* The Intracellular enzymes of *Penicillium* and *Aspergillus,* with special reference to *Penicillium camemberti.* U.S. Dept. of Agric., Bur. Animal Ind. Bull. 120 (1910), 7-65.

(6) *Hamond, J. B.* A Survey of the Walnut Position in England, 1932. East Malling Res. Sta. Ann. Rpt. (1932), 73-8.

(7) *McAlpine, D.* Fungus Diseases of Stone Fruit in Australia and their Treatment. Dept. of Agric., Victoria, March 1902.

(8) *Peyronel, B.* Una nuova malattia del lupino prodotta da *Chalaropsis thielavioides* Peyr., nov. gen. et nova sp. Le Staz. sperim, agrar. Ital. (1916), **49**, 583-96.

(9) *Witt, A. W.* The Vegetative Propagation of Walnuts. East Malling Res. Sta. Ann. Rpt. (1926), 60-4.

(10) *Wormald, H.* Bacterial "Blight" of Walnuts in Britain. Ann. of App. Biol. (1930), **17**, 59-70.

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RASPBERRY BREEDING AT EAST MALLING, 1922-34

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PART I. PRACTICAL ASPECTS.

1. INTRODUCTION.

ALTHOUGH raspberries have been grown in gardens for centuries, only in recent times has there been any systematic attempt at their improvement. Until the middle of the nineteenth century or later, the varieties in cultivation were all, so far as is known, forms of chance appearance, discovered either wild or in gardens. In the last seventy or eighty years much systematic breeding has been done, both in Great Britain and abroad ; amongst English breeders, the nurserymen Rivers, Pyne and Laxton have shown the practical possibilities of such work. Considerable genetical work with raspberries has been done at the John Innes Institution at Merton, and both genetical and practical work at the Long Ashton Research Station.

That much remains to be done, and that there is ample scope for improved methods, is shown by the present situation: the one variety Lloyd George, which is being more planted in England and Scotland than all other varieties put together, is stated to have been found growing wild in a wood. It may be estimated also that of other varieties still more or less planted commercially in the British Isles, quite half are chance forms, found either in gardens or the wild state.

A detailed history of the improvement of the raspberry in both Europe and America, was published in 1925 in "The Small Fruits of New York" (1). Though a few reports on the same subject have since then been issued in America, there is little fresh matter of interest to British readers. The work of Crane and Lawrence (2, 3) in England, whilst of great interest to the geneticist, does not yet provide much guidance to the practical breeder. It is hoped that this report will serve to stimulate interest in the more practical aspects of the subject.

2. OBJECTS AND METHODS OF THE WORK.

Previous reports (4, 5) have shown that when raspberry investigations were begun at East Malling and Long Ashton, varietal nomenclature was in extraordinary confusion. This led to the suspicion, subsequently confirmed in at least two instances, that raisers of new raspberry varieties might be in doubt, or mistaken, about the identity of the varieties they were using as parents. It therefore seemed desirable to begin breeding work with varieties free from " rogues ", and for which a uniform system of nomenclature had been devised.

For the first ten years the chief object of the work was the production of varieties improved in fruit and vegetative characters, the disease factor being largely ignored. With this in view, a search was made for varieties likely to produce a good proportion of seedlings of high commercial merit. The blossoms of nineteen named varieties were bagged and self-pollinated and families raised from the seed thus produced. Subsequently, some of the more promising parents were crossed, either with each other, or with selected seedlings of other parentage ; crosses were also made between some of the best seedlings of different parentage. Selected seedlings of two families have also been " selfed " to produce the F_2 generation.

Mosaic disease soon began to appear amongst the seedlings. In commercial plantations the disease was yearly becoming more serious, and much time was being spent in devising methods of control (6). Since there proved to be a marked variation amongst cultivated varieties in infectibility and susceptibility to the disease, there was clearly a possibility that good fruit and vegetative characters might be combined with low infectibility or low susceptibility. The last two crosses have therefore each included one parent showing low infectibility by the disease ; their susceptibility is still in some doubt, but one at least appears resistant.

Characters of a good commercial raspberry.

The fruit and vegetative characters required in a good commercial raspberry are as follows:

For the retail markets and for canning, the fruit must be large, smooth in outline, " compact " (i.e. not falling apart or " crumbling " when picked), readily parting from the receptacle (i.e. " easily plugged "), and yet not dropping badly, firm enough to travel satisfactorily to market in packages at least as large as 4 lb. chip baskets, and remaining whole when canned or bottled. It must have a good, clear, bright colour, not turning dark when fully ripe, and must be of at least passable quality for dessert. For jam making the fruit need not be so large (large fruit, however, reduces cost of picking), or so brightly coloured: soft fruit is generally preferred, since it " goes down " more in the tubs or barrels, allowing them to hold more weight. For both purposes the fruit should ripen as evenly as possible over a period neither too short, nor too long drawn out. Since different growers and different districts require raspberries of different seasons—early, mid-season, or late—no one variety can satisfy all requirements.

The plant must be a heavy and consistent cropper, adaptable to many soils and climates, and not requiring heavy and frequent manuring ; it must be reasonably vigorous, producing enough canes, but not smothering the fruiting canes by a dense growth ; it must not be very spreading in habit, and the canes must be reasonably free from spines, at least on their upper half. Finally the plant must be resistant to diseases, and to injury by frost, whether to the canes in winter or the blossoms in spring.

These requirements are clearly numerous enough to be difficult to combine in one variety ; no variety at present in common commercial use in England comes within a long distance of satisfying all of them. They are all represented in at least reasonable measure, however, in one variety or another ; breeding on a large enough scale should, in the end, bring them all together. The only doubt is the possibility that some desired characters may be correlated with serious faults ; as yet there is nothing to suggest that this may occur.

Sources from which the desired characters might be obtained by the breeder are as follows (the best are mentioned first)* :

Large fruit: Pyne's Royal, Lloyd George, Preussen, Red Cross, perhaps even Superlative A.

Smooth outline: Burnett Holm, Red Cross, Lloyd George.

"Compact" fruit: Most varieties: Bath's Perfection was often the worst.

Easy "plugging": Most varieties: Red Cross, Devon, Pyne's Royal and some others are said sometimes to offend in this respect. Many seedlings are very bad.

Firm fruit: Baumforth Seedling B, Pyne's Royal, Burnett Holm. The firmest variety ever grown at East Malling is rarely met with—Penwill's Champion.

Bright colour: Preussen, Bath's Perfection, several American varieties.

Not turning dark: Burnett Holm, North Ward, Preussen, Mitchell's Seedling, Norfolk Giant.

High dessert quality: Norwich Wonder A, Preussen, Baumforth B, Hornet A.

Soft fruit (for jam): Baumforth A, Hornet A.

Heavy and consistent cropping: Lloyd George, Baumforth A, probably Norfolk Giant. Red Cross often meets this requirement but appears in some conditions to have "off" years.

Vigorous growth: Norfolk Giant, Baumforth A, Preussen.

Erect habit: Preussen, Pyne's Royal, Bath's Perfection.

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* The reader will understand that recently introduced varieties are seldom as yet well enough known to be here included.

Freedom from spines: Most varieties, except in first year or two. Red Cross sometimes offends in this respect; Baumforth A is definitely bad.

Resistance to disease: MacLaren's Prolific, Baumforth A, perhaps Pyne's Royal, Red Cross.

Hardiness: Little known; in Scotland Lloyd George is found to be one of the best.*

With so many characters to be observed, the writer has found it useful, after propagating many seedlings, to employ a score card, at least for fruit characters. For such plant characters as cropping and disease resistance this is hardly possible; nothing short of a carefully planned trial will suffice. For fruit characters the following scale of points has been found helpful:

Size	15	points.
Smooth outline	5	,,
"Compactness" (not crumbly, small drupelets)	10	,,
Firmness	20	,,
Ease of "plugging"	10	,,
Colour: Brightness	15	,,
Not turning dark	15	,,
Dessert quality	10	,,
	100	,,

Longer experience may no doubt lead to the modification of this scale in some respects. But in any case, it cannot be used rigidly; the seedling receiving the highest total marks may not be commercially the best. Several seedlings, for instance, have come very near top marks in every point but one—their fruit was much too dark coloured when fully ripe. A very low mark for this or any other character is enough to condemn any seedling, no matter how good it may be in other respects. Nevertheless, marking the fruit of seedlings according to this scale has materially helped in eliminating the poorer forms.

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* The frost of May 17th, 1935 has revealed wide differences in blossom hardiness. Only one British variety of any economic importance—Improved Beehive—showed little injury. On the other hand, only one American variety—Herbert—out of six was severely damaged. Amongst seedlings also wide differences are shown. The hardiest family amongst those still growing in the seedling bed was No. 24, Owasco, selfed; one of the remaining plants was badly damaged. Of families of European parentage showing one or more uninjured, open blossoms in early June varied from 16 per cent. in Family 33 (Preussen × Seedling) to 56 and 55 per cent. in Families 34 (Preussen × Baumforth A) and 35 (Preussen × MacLaren's Prolific) respectively. Propagated seedlings of the same family showed at the same time a variation from over 95 per cent. to under 50 per cent. of the open blossoms killed.

Methods Employed.

Since the chief object of the work was the raising of improved varieties, no more than ordinary care was taken to prevent errors. The plants used as parents were growing in the East Malling variety collection or the seedling trial plots, under ordinary field conditions. The flowers were always bagged, including those from which pollen was obtained for crosses ; flowers in bags damaged enough to admit insects were not used.

It was found advisable, after some little experience, to select for bagging the blossoms of a plant not showing any symptoms of *Verticillium* wilt ("blue stripe wilt"), such as the growth of buds on one side only of the cane. No precautions were taken, except in the case of Families 31 to 35 (see Table I), to select for this purpose plants free from mosaic disease. Nine of the named varieties used as parents are in fact believed to be unobtainable free from this disease.

The fruits were collected when over ripe and allowed to dry. The seed was then rubbed out, and sown at once in partially sterilized (steamed) soil in pans, which were kept covered up under the bench of a greenhouse until germination began.

No record was made of the number of seeds sown or the number which germinated. Except in the earlier families, however, germination was generally fair. Family 14 (Table I), Baumforth B selfed, was an exception. This was the third attempt to produce a selfed family from this variety ; the earlier attempts gave no germination at all. All the other families, from 8 to 15, raised at the same time and in the same conditions as Family 14, gave much better germination ; of Families 9, 10 and 12 few seeds were available.

The seedlings, when large enough, were usually potted up in unsterilized soil. It is just possible that in one or two cases the potted seedling died, and a self sown seedling took its place. That this can occur has been shown by the rare presence of blackberry seedlings, growing with the raspberry seedlings in the pots. The pots were examined at intervals, and wherever the plants had died, they were removed. The chances of any error having arisen in this way are very remote.

Some of the seedlings belonging to Families 32, 34 and 35 were planted straight into the field from the seed pans. It was found that when weather conditions are favourable, such seedlings grow quite as well as similar seedlings planted at a later stage from pots. Damp weather is essential for success. When given ordinary care, if the weather is not too dry, the plants will soon start into rapid growth. The method has the disadvantage that it is seldom possible at this early stage to exclude unwanted plants such as yellow fruited and sterile forms ; many of these can easily be identified by the time the pot plants are large enough for transplanting.

TABLE I.

Seedling Families raised, showing Number Selected.

	Index Number.	Year of Germination.	Parent or Parents.	Number of Seedlings. *Red Fruited Not Sterile.*	Number of Seedlings. Marked "*Promising*".	Number of Seedlings. Propagated.
Named Varieties "Selfed".	1	1922	Bath's Perfection Selfed	40	1	1
	2	,,	Superlative A ,,	30	7	7
	3	,,	Pyne's Royal ,,	74	43	22
	4	,,	Semper Fidelis A ,,	84	12	9
	5	,,	Norwich Wonder A ,,	8	0	0
	6	1924	Lloyd George ,,	92	28	17
	7	,,	Baumforth Seedling A ,,	74	8	3
	8	1925	Norwich Wonder A (as "Fillbasket" A) ,,	56	11	7
	9	,,	,, ,, (as "Storrie's Invinc.") ,,	6	1	0
	10	,,	,, ,, ,,	19	2	1
	11	,,	Hornet A ,,	35	2	1
	14	,,	Baumforth Seedling B ,,	9	1	0
	15	,,	Northumberland Fillbasket Thornless ,,	71	9	2
	16	1926	Red Cross ,,	38	2	1
	17	,,	North Ward ,,	87	2	1
	18	,,	Mitchell's Seedling ,,	61	3	0
	19	,,	Red Antwerp A ,,	21	0	0
	20	,,	Black Antwerp A ,,	17	1	0
	24	1930	Owasco ,,	13	0	0
	25	,,	Burnett Holm Seedling ,,	25	3	2
	26	,,	Winkler's Seedling ,,	28	0	0
	31	1932	Preussen ,,	65	0	0
F_2 Families	13	1925	F_2 Pyne's Royal (Family 3, No. 5) ,,	66	1	0
	28	1930	F_2 Family 23, No. 38 ,,	22	3	3
	29	,,	F_2 Family 23, No. 42 ,,	8	0	0
	30	,,	F_2 Family 23, No. 56 ,,	19	2	2
Crosses	12	1925	Superlative A ♀ × ♂ Seedling (2/14)	6	2	2
	21	1928	Pyne's Royal ♀ × Lloyd George ♂	128	26	8
	22	,,	Family 6, No. 23 ♀ × Family 3, No. 72 ♂	101	30	8
	23	,,	Family 3, No. 10 ♀ × Family 6, No. 86 ♂	100	27	9
	27	1930	Pyne's Royal ♀ × Winkler's Seedling ♂	12	1	1
	32	1932	Preussen ♀ × Lloyd George ♂	269	45	19
	33	,,	Preussen ♀ × Family 23, No. 50 ♂	193	28	13
	34	1933	Preussen ♀ × Baumforth Seedling A ♂	45	—	—
	35	,,	Preussen ♀ × MacLaren's Prolific ♂	128	—	—

Growth and Fruit-bearing of Young Seedlings.

The height which the plants reach in their first year naturally varies with seasonal and local conditions, as well as with the inherent vigour of the plant. In good conditions, and in a season not too dry, a vigorous family may average eighteen inches or more in the year of germination ; individual plants at East Malling have frequently reached three feet. In their second year, vigorous individuals are usually as much as five feet in height, and may reach six feet or more.

No definite evidence amongst raspberry seedlings of a "juvenile period" has yet been found at East Malling. Plants which reach a height of less than one foot in their first year frequently fail to produce blossoms in their second year ; in such cases the growth from the old cane may be purely vegetative, sometimes surviving a second winter and blossoming in the third year, or it may produce flower buds which shrivel or fall before they open. But even where plants bear fruit in their second year, it has been found by experience that the quantity of fruit is no guide whatever to the cropping capability of the seedling. Vigorous plants which bear only a few scattered fruits in their second year, may, in their third, produce an unusually heavy crop. Perhaps there may here be a trace of juvenility.

3. THE COMPARATIVE VALUE OF DIFFERENT VARIETIES AS PARENTS.

Table I gives a complete list of all the seedling families so far raised. Twenty-two families were raised by self-pollinating the flowers of nineteen named varieties ; four were F_2 families, produced by self-pollinating the flowers of seedlings in earlier families ; and the remaining nine were the results of various crosses. Column 4 gives the number of seedlings available for selection, i.e. the number surviving to fruiting age ; yellow and "apricot" forms are here excluded, as are also sterile forms with male or neuter flowers, and suppressed pistils. Families 2, 5, 8, 9, 10, 11, 12, 16, 18, 20 and 26, in which male sterile forms occurred, almost certainly contained some female plants, with suppressed stamens. Some of these, described as "weak females", are said by Crane and Lawrence (3) to bear very poor fruit, consisting of only a few scattered drupelets. Whilst no attempt has been made to record the number of plants in the above mentioned families which had suppressed stamens, most of them, and particularly Family 16, included some plants with fruits similar to the weak females described by these authors. The presence of females would obviously reduce (in some cases by 50 per cent.), the number of fruit-bearing plants in these families from which promising selections might be made.* Further reference to this point is made below.

* Female plants are clearly not wanted as commercial varieties, since they would have to be interplanted with a pollinator.

Columns 5 and 6 give the number of seedlings in each family which appeared promising in the original bed, and the number subsequently propagated. It will be seen that the families varied greatly in the proportion of their individuals thought worthy of further observation. Unfortunately the numbers are not strictly comparable. Families 1 to 5 were planted out in good soil in a sheltered place, and grew extremely well. Numbers 6 to 23 had a piece of moderately good soil, rather exposed to winds from every direction ; they grew moderately well. The remainder had to make do with a notoriously poor plot of soil, less sheltered than the first but more than the second. In spite of this handicap some of the later families, particularly 32 and 33, have grown very well ; many plants were six feet in height or more after two seasons' growth from germination.

In spite of these unavoidable differences in treatment, it is certain that some parents, when selfed, have given a much larger proportion of good seedlings than others, and this although the last of the selections from selfed families up to No. 20 were discarded at the end of 1933. It will be worth while, from the data collected, to try to find answers to three questions: (i.) Is it possible to obtain from a selfed family forms commercially superior to the parent ? (ii.) Are the best named varieties at present in cultivation likely to give, when selfed, a larger proportion of promising seedlings than others ? (iii.) Will parents which give the largest proportion of promising seedlings when selfed behave in the same way when crossed amongst themselves ?

Characters of Selfed Families and Their Parents.

A brief description of some of the parents, and their progeny, and particularly of the faults which led to the discarding of so many promising seedlings, will provide material for answers to these questions.

The parents from which most was expected, when selfed, were Pyne's Royal, Lloyd George, Red Cross, North Ward and Preussen. PYNE'S ROYAL is still one of the largest fruited varieties in cultivation. Individual fruits of other varieties may often equal or surpass its best in size ; but few, if any, others produce such uniformly large fruits, with so few small. Yet it has very serious faults. Though usually tall enough in growth, it rarely produces enough canes to make a reasonably thick row ; as a rule the number of canes is too small to allow of heavy cropping, and the rows soon become gappy. The colour of the fruit is rather poor, being usually dull and turning much too dark when fully ripe. The fruit is usually firm enough, in spite of its great size, but it is said sometimes to be difficult to "plug". Its rather acid flavour, though relished by some, is not such as to make it a really popular dessert fruit. Finally its fruit has a rather uneven shape.

Its selfed seedlings (Family 3) as a group, well illustrate its good and bad points. No other selfed family so far raised has approached them in average fruit size ; and the canes were usually tall enough, even after some years of propagation. But nearly every propagated seedling exaggerated the parent's faults. Very few bore fruit more brightly coloured than the parent's, and nearly all turned even darker than the parent's when fully ripe. A few after propagation produced a satisfactory number of canes ; but here the fruit did not maintain its size. No. 72 (used as the male parent of Family 22), which had the largest fruit of all, was discarded mainly because it would not produce more than one or two canes per stool. Many others were in this respect as bad. Several seedlings were described again and again as " impossible to plug ", and many others were by no means easy. Of the latter, one was No. 5, self-pollinated to produce Family 13. This family, by far the most uniform so far raised (the only F_2 from a selfed family), contained hardly an individual free from the " difficult plugging " fault. Nearly all also resembled their immediate parent in having a disagreeable flavour. Whilst in fruit shape No. 5 (like its selfed progeny) was distinctly better than its parent, many others exaggerated the fault of Pyne's Royal, having fruit noted as " warty ", or with a constriction near the middle. A considerable number also, unlike the parent, produced " crumbly " fruit, with large drupelets.

LLOYD GEORGE, though very large fruited, is almost as different from Pyne's Royal in other respects as it could well be. It usually grows tall enough when young, but is apt in the poorer soils to fall off in vigour, even apart from the effect of mosaic disease. It produces immense numbers of new canes, which, unless drastically reduced, are apt with their heavy foliage to smother the weaker fruiting canes, and make the fruit difficult to pick. Its fruit, though nearly all very large in the first year or two of cropping, becomes much more uneven in size, and often mostly small, in later years. (This may be in part due to mosaic disease.) The fruit is reasonably brightly coloured (though not approaching that of Bath's Perfection and Preussen), but when fully ripe it turns almost as dark as that of Pyne's Royal. In a warm picking season it is necessary for canning to pick the fruit every day ; otherwise far too much has to be thrown out to maintain the quality of the product. Its flavour, though more often appreciated for dessert than that of Pyne's Royal, leaves a good deal to be desired. The present writer knows of no case where Lloyd George, or any of its selfed seedlings, have been difficult to " plug ". The variety is redeemed by its remarkably heavy and consistent cropping.

Here again the selfed seedlings (Family 6) more often exaggerated the faults than the merits of the parent. Of those propagated, not one failed to produce abundant canes, and most produced far too many. In the original seedling bed the selected plants were tall enough, but after propagation they all became too weak for commercial use ; several produced few canes more than eighteen inches high. Had the best of them concentrated the same length of cane growth in one-third the number of canes, they would undoubtedly have been worthy of further trial.

In this family a much smaller proportion bore outstandingly larger fruit than in No. 3 (Pyne's Royal selfed). Yet many were at first quite as large as the parent, and several were larger. After propagation, however, not one could fairly be said to be as large as the parent (which, by the way, was growing in similar conditions near by). In fruit colour they were far superior to those of No. 3. None was outstandingly brightly coloured, but many were good, and several were not seriously dark coloured when fully ripe. As in Family 3, many of the seedlings bore "crumbly" fruit, a fault not commonly noticed in the parent. This fault, which curiously appeared unexpectedly in these two families, was even more evident in Family 21, raised by crossing the two parents.

RED CROSS at its best is not far behind the foregoing varieties in fruit size. The plants are usually vigorous, producing taller canes than either Pyne's Royal or Lloyd George, and somewhat less in number than the latter. The canes are distinctly spreading in habit of growth, and are often more spiny than is desirable in a commercial raspberry. In a windy place these spreading and spiny canes are apt to damage a large proportion of the fruit. When well grown the fruit is of exceptionally good appearance, smooth conical in shape, and reasonably brightly coloured ; it has less tendency than either of the foregoing varieties to turn dark when fully ripe. Though difficult "plugging" has not been noticed at East Malling, a few growers have reported this fault, which appears in this case to be seasonal, and is perhaps worse in dry years.

Family 16, Red Cross selfed, was unexpectedly disappointing. Apart from the fact that out of seventy-one plants, thirty-three were either yellow fruited or sterile, leaving only thirty-eight from which to select, these thirty-eight plants included not a single form likely to be superior to the parent, except, perhaps in cropping. The one plant propagated (No. 52), both before and after propagation bore most remarkable crops ; but the fruit, though of good colour, was never up to the size of the parent's, and was in addition tasteless.

NORTH WARD seemed for a time one of the most promising varieties in the East Malling collection. Longer experience has shown that, at least in the East Malling soil, it needs heavy feeding. Lack of feeding reduces both the crop and the fruit size very materially. At its best it is excellent in nearly every respect, though in fruit size it may still be slightly behind Pyne's Royal, Lloyd George and Preussen. It excels particularly in vegetative characters and fruit colour. The plant is vigorous, usually healthy, making enough canes and seldom too many, and the foliage is not heavy enough to shade the fruit seriously. The fruit is rather pale coloured and has the advantage, like that of Burnett Holm, of not turning dark.

North Ward selfed, Family 17, was even more a disappointment than Red Cross selfed. Out of seventy-four red-fruited normal seedlings (seventeen others were deficient in chlorophyll and bore practically no fruit) only one gave enough promise for propagation, and even that failed to live up to its promise. The fault was almost invariably small crumbly fruit. The plants as a rule grew well, and might have cropped well had the fruit been large enough. The pale coloured fruit was inherited by very many of the seedlings ; hardly any turned seriously dark when fully ripe.

PREUSSEN, introduced from Germany several years ago, seemed at first an improvement on any variety in cultivation. The growth was immensely vigorous, the rather sparse canes stiff and upright, and reasonably free from spines. The crop appeared likely to be very heavy (available records are unsatisfactory) ; the fruit was very large, nearly round and smooth, of magnificent colour, and equal to the best in dessert quality. Experience has shown that the fruit is often too soft for the dessert or canning trade, and worse still, that the plant is highly susceptible to mosaic disease. Its infectibility is not yet known with certainty, but it has been difficult at East Malling to keep a small stock of it apparently free from the disease. Occasional faint leaf mottlings may indicate that the stock is not even now entirely free.

Family 31, Preussen selfed, was again a great disappointment. Though the seed only germinated in March 1932, three years' growth and one year's attempt at cropping have been enough to condemn the whole family forthwith. Out of sixty-five red-fruited plants, only four have grown as tall as the average in Families 32 and 33 (planted at the same time), where Preussen was the female parent, and these four made very few canes, and in addition, bore poor fruit. The bulk of the plants are obviously too lacking in vigour to be of any value. Though the fruit of nearly all was of fine colour and dessert quality (several, however, unlike the parent, turned dark when fully ripe), it was in most cases small and very "crumbly".

A few other varieties and their selfed seedlings are worth a brief mention. SEMPER FIDELIS A bears moderate sized acid fruit ; its canes are very susceptible to cane spot. Those of its selfed seedlings propagated (Family 4), were the largest fruited of the family ; some probably averaged larger than the parent. None, however, was large enough for modern requirements. The plants grew well, but every one proved to be very susceptible to cane spot. The majority bore acid fruit ; few were as acid as the parent, and one or two were moderately sweet.

BAUMFORTH SEEDLING A is exceptionally vigorous and healthy, with moderate sized, round, soft fruit, extremely acid. Family 7, Baumforth A selfed, consisted mainly of forms very similar to the parent, particularly in fruit characters. The three propagated appeared at first to exceed the parent in fruit size ; their fruit was firmer and less acid. After propagation, though they maintained their vigour, their fruit became too small.

NORWICH WONDER A is an old variety of the same "curled leaf" type as Superlative A and Pyne's Royal. It is seldom vigorous (perhaps partly owing to mosaic disease), and produces hardly more canes than Pyne's Royal. Its fruit is large, one of the earliest to ripen, dark coloured even before fully ripe, and best in dessert quality. Family 8, Norwich Wonder A selfed (together with the small and similar lots in 5, 9 and 10), was quite exceptionally variable in every character. Even when yellow fruited and sterile forms are omitted (amounting in the four families to fifty-six plants out of one hundred and forty-three), the remainder showed an amazing range in vigour, foliage character, size and colour of fruit, and other fruit characters. A few plants bore fruit almost equal to the best in Family 3 (Pyne's Royal selfed), whilst others were extremely small. A few, all of which happened to be small fruited, produced as many canes as Lloyd George, whilst others, including all those propagated, could not be induced to make enough canes. The fruit of most of those propagated turned too dark coloured when fully ripe. The raiser of Pyne's Royal, in a letter to the present writer, stated that this variety was originally found as a small self-sown seedling under a row of Norwich Wonder. It is perhaps worth suggesting, in view of this statement and the experience with Family 3 mentioned above, that there may be at least a partial correlation between fruit size and "spawn" production, large fruit going with few canes, and vice versa. Records available here are not full enough to provide definite evidence.

Family 15, Northumberland Fillbasket Thornless selfed, was raised chiefly to show how far the spine-free character appeared in the progeny. This is referred to below. Here it may be noted that though the parent appears, in East Malling conditions, to have no commercial value, the two seedlings propagated might have had considerable value, being apparently much improved in cropping and fruit size. They were discarded because they were still far behind the best seedlings of the crossed families.

With these facts in mind, we may now hazard answers to our first and second questions.

(i.) Although very many individuals in families raised by selfing named varieties have exaggerated the faults of their parents, and some have had faults not evident in their parents, it is clear that individuals superior to their parents *can* in some cases be raised in this way. In spite of their faults, several seedlings in Family 8 (Norwich Wonder A selfed), were almost certainly preferable to their parent for commercial purposes; the same is true of the two seedlings in Family 15 just mentioned. Both these parents are, however, of little value. In families raised by selfing varieties of high value, we have not so far found one seedling which on balance could confidently be said to be superior to its parent. Clearly, to obtain improved forms in this way, one would have to raise very large families, thus greatly increasing the work of looking after and observing the plants.

As commercial forms, then, selfed seedlings are not encouraging. We shall see presently that they may be of great service as parents of crossed families.

(ii.) It is fairly clear that we cannot judge from the commercial value of the parent what proportion of its selfed seedlings is likely to be of high commercial value. We have the very disappointing families raised by selfing Red Cross (16), North Ward (17), and Preussen (31), where hardly a single seedling was considered worthy of further observation; and on the other hand, the families raised by selfing Norwich Wonder A, which gave much better results than could have been anticipated.

(iii.) To answer our third question, whether parents which give the most promising families when selfed will also behave in the same way when crossed, we have the data from Families 21 and 32, where Lloyd George, as pollen parent in each case, was crossed with a "good" parent, Pyne's Royal, and a "poor" parent, Preussen, respectively. Family 32 is as yet too young to have given full results; many plants gave a small amount of fruit in 1933, and most gave a full crop in 1934. It is probable that another year's observations will somewhat increase the number of plants thought promising or worthy of propagation. Family 21, on the other hand, is now discarded, except for two or three of the propagated plants still under observation.

From Family 21 (Royal × Lloyd George) there have been selected 21·5 per cent. of plants considered "promising"; 6·5 per cent. have actually been propagated. From Family 32 (Preussen × Lloyd George) we already have 19·7 per cent. marked "promising", and 8·3 per cent. propagated ("yellows" and "apricots" are, of course, excluded in each case). Thus Family 32 (Preussen ♀), with the handicap of very poor soil only partly balanced by improved shelter, has already given results fully equal to Family 21 (Pyne's Royal ♀), and is almost certain to exceed the latter after another year. Preussen, the "poor" parent, will have justified its crossing with Lloyd George better than Pyne's Royal, the "good" parent. The fact that practically all the seedlings selected from Family 21 have now been discarded cannot be counted against that family ; it is in any case partly due to the intervention of mosaic disease. Another four years may see the same fate overtake the selected seedlings of Family 32.

Characters of Crossed Families and Their Parents.

Family 21, the cross between Pyne's Royal and Lloyd George, and Families 22 and 23, each raised by crossing a selfed seedling of one of these varieties with a selfed seedling of the other, were all treated identically throughout, and thus are strictly comparable. Each family has certain characteristics, quite plainly derived from its parents.

The named varieties have already been described. Each is approximately midseason in ripening, Pyne's Royal perhaps a little the later. Family 21, raised by crossing them, was distinctly more variable in almost every respect than Families 22 and 23, even apart from the fact that No. 21 contained nearly 25 per cent. of "yellows" and "apricots", whilst 22 and 23 had none. In number and character of canes, the great majority of Family 21 resembled Lloyd George, their pollen parent, more closely than Pyne's Royal ; not one failed to produce far more canes than the latter. In foliage character there was great variation, from an approach to the "curled leaf" type of Pyne's Royal to leaves almost as "flattish" as those of Lloyd George. Similarly in fruit characters, there was a great variety of shapes ; colour varied from brighter than Lloyd George to duller than Pyne's Royal, and its depth from fruit really "pale" when over-ripe to fruit almost black when ripe. A majority of the seedlings bore fruit with a strong tendency to "crumble", much more than either parent, and more, it was estimated on the average, than Families 3 and 6, raised by selfing the parents. Nevertheless, there were several seedlings in Family 21 with fruit quite as "compact" as that of either parent. Many were condemned on account of their soft squashy fruit. Finally, in flavour and dessert quality there was a surprising variation, though none was actually disagreeable, like those already mentioned in Family 13 (F_2 Pyne's Royal selfed).

The parents of Family 22 were described as follows: No. 6/23, seed parent, one of the best of its family (Lloyd George selfed), in size, colour and general appearance of fruit, somewhat earlier in ripening than its parent. No. 3/72, pollen parent, already mentioned as having largest fruit of its family (Pyne's Royal selfed). Immense fruit exceptionally tasteless ; less dark coloured when fully ripe than most of its relatives. Both had slightly more tendency than their parents to produce "crumbly" fruit ; but neither was bad. Fruit of both about as firm as that of their parents.

Family 22 closely follows its immediate parents in all these respects, except that the fruit was seldom tasteless. Hardly one was later than midseason in ripening ; several were distinctly early. In fruit colour nearly all were fairly presentable, and hardly any turned very dark when fully ripe. In spite of the tendency of the fruit of both parents to "crumble", far less plants carried this fault than in Family 21 ; and there were also far less plants than in Family 21 with soft and squashy fruits. There was in this case a clear gain in several directions, and no loss, through crossing two selfed seedlings instead of their parents.

Family 23 resulted from crossing two seedlings described as follows: No. 3/10, seed parent, second in fruit size to 3/72 in its family (Pyne's Royal selfed) ; later ripening even than Pyne's Royal ; fruit less brightly coloured and turning much darker than that of 3/72 ; sometimes difficult to "plug". No. 6/86, pollen parent, before propagation almost as good in fruit characters as 6/23, and better than most of its family (Lloyd George selfed) ; deteriorated after propagation, fruit became smaller and "crumbly" ; fruit when fully ripe somewhat darker than 6/23 or even than Lloyd George ; midseason in ripening.

Family 23 again closely follows its immediate parents. Few plants were earlier than midseason in ripening and most were later, like the seed parent. Nearly all turned much too dark when fully ripe ; only an occasional plant bore fruit remaining reasonably pale coloured. And many more in this family than in Family 22 had "crumbly" fruit, like that of their pollen parent. In this case, owing to the dark coloured and "crumbly" fruit, there was not much to choose between the cross of the seedlings and that of the parents.

It might be thought that the greater variability of Family 21 would give more scope for the selection of outstanding forms than the greater uniformity of 22 and 23. This, however, was not so. Table I shows that a somewhat larger proportion of the plants in both 22 and 23 than in 21 were marked "promising" ; the same is true of plants subsequently propagated. Of the twenty-five plants propagated from the three families together, four were eventually chosen for extended trial ; three of these come from Family 22, and one (one of the few "pale" fruited forms), from Family 23.

The conclusion to be drawn from this comparison of the three families, 21, 22 and 23, may have an application beyond the limits of raspberry breeding.

Much better success may be expected from crosses of selfed seedlings of different parentage than from the cross of their parents. But the seedlings must be most carefully selected. Selfed seedlings, being less heterozygous than their parents, will pass on their characters, whether merits or faults, much more definitely than will their parents. Any such seedling which has the same fault as its parent in a degree no greater than that of the parent is extremely undesirable for crossing ; at the very least we must make sure that if one seedling has such a fault the other, to be crossed with it, is free from the same fault. Largely through lack of attention to this point, Family 23 was much less valuable than it might have been. It is equally clear that the selfed families must be large enough to allow ample scope for the selection of satisfactory forms.

Family 27 was too small to provide much information, except in two directions, vigour and infection by mosaic. Its parents, Pyne's Royal and Winkler's Seedling, are closely similar in cane and foliage characters, except that the latter makes a considerable number of canes ; but they are very different in fruit characters. Winkler's Seedling has a small, very bright coloured fruit, turning only slightly dark when fully ripe ; its fruit is usually as firm as that of Pyne's Royal, but has more tendency to " crumble ". Both varieties are believed to be always infected with mosaic disease, but both appear to be little injured by it. Of the eighteen plants in the family, six were " yellows " or " apricots ". All were distinctly more vigorous, and made far more canes, than either parent. Few bore fruits appreciably larger than that of Winkler's, and all had the " crumbly " tendency strongly developed. By the fifth summer after germination (1934), four plants had developed obvious mosaic symptoms, much more definite than those usually shown by either parent ; three or four more were under suspicion. This family appears as a whole to have been more readily infected with mosaic than any other family so far raised. The infected plants were not allowed to remain long enough to show their susceptibility to the disease definitely ; but it appeared probable that two or three of them were dwarfed by it.

It may perhaps be suggested that the possibility of the seed transmission of virus in raspberries cannot yet be altogether ruled out, though no clear evidence of it has yet been seen.

The later crossed families (32-35) are already interesting, but require longer study before any definite report on them can be made. (Scarcely half the plants in 34 and 35 have yet borne enough fruit for satisfactory description.)

Families 32 and 33 (Preussen × Lloyd George, and Preussen × Seedling 23/50), whilst each showing certain family characteristics, seem fairly similar in the extent of their variation. (Family 32, however, included over 20 per cent. of " yellows " and " apricots ", whilst 33 had none.) Since the seed parent was the same for each family, and since the pollen parent of Family 33 was the product of a cross between seedlings of widely different parentage, and was therefore very heterozygous, this is only what might be expected.

Family 32 (Preussen × Lloyd George) contains many plants with leaves resembling those of Lloyd George, and few with an approach to the more convex and rugose leaves of Preussen. In fruit colour, very few approached the seed parent, Preussen, in brightness, but many were brighter than Lloyd George; some few were duller. The depth of colour when fully ripe varied far beyond the limits of the parents, from really "pale" fruit to "nearly black"; in numbers, those with fruit "too dark" and "not too dark" were nearly equal. Although both parents, when selfed, produced an unexpectedly large proportion of plants with "crumbly" fruit, their cross produced far less of this type than did that of Pyne's Royal and Lloyd George (Family 21).

The seedling used as pollen parent of Family 33 (Preussen × 23/50) was selected for its large firm fruit, which had little tendency to "crumble". Its fruit was known to turn too dark coloured when fully ripe; it was hoped that in a cross with Preussen, which is comparatively free from this fault, the fault would not be troublesome. Although this hope was not realized, Family 33 has been very valuable in showing something of the inheritance of this fault.

The family (33) contains many plants with leaves as convex and rugose as those of Preussen, and a few approaching Pyne's Royal; very few were at all flattish, like those of Lloyd George. (In this respect the male parent was intermediate between Lloyd George and Pyne's Royal, its ultimate progenitors.) In cane character it was very similar to Family 32, but most canes were somewhat darker coloured. In spine character, however, whilst 32 (without its "yellows" and "apricots"), contained only plants with red spines, about one-third of the plants in 33 had tinged spines. As soon as spine colour and fruit colour were compared, it was found that there was a definite, though incomplete, correlation between tinged spines and fruit not turning dark, on the one hand, and red spines and dark fruit on the other. The complete figures are given in a later section; here it may be said that the exceptions are few, as the following particulars will show. Of plants fruiting in 1934, 60 had tinged spines, and of these 21 (35 per cent.), were marked "promising"; 19 of these 21 bore fruit definitely not too dark coloured for market, and 2 were doubtful. Of 132 plants with red spines, 16 (12 per cent.) were "promising", but only 6 of these were definitely not too dark coloured; the other 10 must be watched for another year, and may then prove to be too dark.

Nevertheless, had all the plants with red spines been discarded in the pot stage, as might easily have been done, two quite exceptionally promising seedlings would have been lost. The correlation between spine colour and fruit colour was not in this family close enough to allow the breeder to act on it safely. At the same time, it is perhaps open to question whether it might not have been better to raise a much larger family and plant out only those plants with tinged spines. Had this been done, the same amount of effort might have yielded forms still more promising than any now available. The practical breeder does not want to grow more useless plants than necessary.

Families 34 and 35 were raised primarily for an attempt to study the inheritance of low infectibility by mosaic disease. Whilst no opinion can yet be expressed as to the probable proportion of seedlings promising in fruit and vegetative characters, it can already be said that these families contain some plants of much promise.

One plant in 34 and two in 35 have already been removed on account of mosaic infection. Since varieties with low infectibility are uncommon, the character is probably complex, and may not appear in seedlings before the F_2 generation. With the mosaic situation becoming yearly more serious, it is felt that any assistance breeding can provide will be invaluable, even at the cost of many years' work.

PART II. GENETIC ASPECTS.

Crane and Lawrence (3) have reported in considerable detail the inheritance of sex, colour, and hairiness in raspberries. Five families raised at East Malling duplicate families raised by these authors ; only two, however, are fully reported by them. The remainder are distinct. East Malling results bear out, as far as they go, the conclusions of these authors ; in certain directions they also supplement them. In addition, certain forms not mentioned by these authors have appeared at East Malling.

Inheritance of Colour—Spine and Fruit Colour.

Crane and Lawrence conclude that colour in raspberries is due to two factors, P and T. In the absence of T the spines are green and the fruits yellow or " apricot ". T without P gives tinged spines and red fruit ; PT gives red* spines and red fruit. At the time their report was published, they had not completely cleared up the relationship of the various fruit colour forms, such as apricot, and pale and dark reds. They suggested that in the presence of P and absence of T the fruit may be apricot ; and they point out that certain cultivated varieties with tinged spines have pale red fruit.

Results in the present work confirm their implied conclusion that colour inheritance is not really so simple as this. Whilst the yellows and apricots have seldom been carefully observed, and in later families have been discarded in the pot stage, several examples have been seen of a form, having green spines, intermediate between apricot and red. Such fruits, even before they become over-ripe, have a distinct reddish tinge, and might almost be mistaken for a very pale red form.

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* In a former report (5) the present writer used the terms " pale " and " dark " in describing spine colour ; Crane and Lawrence's terms " tinged " and " red ", seem preferable and are here used.

Further, several families which contained only plants with red and green spines, and particularly the crossed Families 21, 22 and 32, have had several red spined plants with distinctly pale red fruit. Family 33 has plants with both red and tinged spines, but none with green; this takes us a step further. It was at once obvious here that most of those with red spines had dark fruit, and most of those with tinged spines had pale fruit, but the other two combinations also occur. Table II gives the complete figures (a few plants were removed before they fruited on account of mosaic disease).

TABLE II.

Spine and Fruit Colour.

	Spines tinged.		Spines red.	
	No.	%	No.	%
Fruit "pale"	3	5·0	0	—
"not dark"	28	46·7	5	3·8
"not very dark"	21	35·0	6	4·5
"rather dark"	6	10·0	36	27·3
"dark" or "too dark" ..	1	1·7	44	33·3
"very dark"	1	1·7	36	27·3
"nearly black"	0	—	5	3·8

The fruit colour was judged entirely by eye, without any fixed standard for comparison; owing to the considerable variation in time of ripening amongst the plants, some may have been entered in one grade higher or lower than they should have been. Experience over several seasons has shown, however, that it is very rarely necessary after one year's observations to transfer any plant to a grade more than one place paler or darker. Even if the fruits described as "not very dark" and "rather dark" are ignored, we still have two plants with tinged spines and dark fruit; and we have five plants with red spines and "not dark" fruit. The latter combination occurred, as we have seen, in several other families; the former was much less frequent, but an approach to it occurred in Families 7 (Baumforth A selfed), and 17 (North Ward selfed), and in Family 31 (Preussen selfed) several plants with tinged spines had fruit which could only be described as "dark". The presence of these forms seems to suggest the linkage of two colour factors other than P, with some crossing over.

In other respects, Crane and Lawrence's results in spine and fruit colour are almost always borne out. As in their work, there is some deficiency of green spined forms in Lloyd George selfed, and a smaller deficiency in Pyne's Royal × Lloyd George. Pyne's Royal selfed, however, which in their work gave some excess of green spined plants, here gave a large deficiency, 74 red, 4 green (expectation 58·5 red and 19·5 green). Nearly every variety selfed also gave some deficiency of greens; the largest was in Baumforth A selfed, with 77 tinged, 3 green (expectation 60 tinged, 20 green). One variety, Winkler's Seedling, when selfed gave an excess of greens, 12 red, 9 green (expectation 15·75 red and 5·25 green). The still smaller family resulting from the cross Pyne's Royal × Winkler's also gave an excess, 12 red, 6 green (expectation 13·5 red, 4·5 green). Both results may be accidental.

Three varieties when selfed gave no green spined plants—Semper Fidelis A, Hornet A and Owasco (Family 24, Owasco selfed, however, consisted of only thirteen plants, not enough for certainty). None of these has been used in crossing.

Superlative A selfed, as in Crane and Lawrence's work, gave an excess of both red and tinged; Norwich Wonder A selfed, however, gave a small deficiency of tinged as well as green, and an excess only of red. Since Crane and Lawrence observed a smaller excess of tinged from the latter than from the former, the two results are to this extent in the same direction.

The only other red spined varieties from which selfed families of any size were raised were: Semper Fidelis A (4), all red; Hornet A (11), 31 red, 16 tinged (expectation 35·7 red, 11·3 tinged); and Mitchell's Seedling (18), 42 red, 19 tinged, 14 green (expectation 42·19 red, 14·05 tinged, 18·75 green). These last two, therefore, both gave some excess of tinged.

All varieties with tinged spines gave, when selfed, as expected, only tinged and green.

The larger crossed families in no case gave more than two colour types. Families 22 and 23 (Seedling × Seedling) gave all red; 33 (Preussen × Seedling) red and tinged; 21 (Pyne's Royal × Lloyd George) and 32 (Preussen × Lloyd George) red and green; and 34 (Preussen × Baumforth A) and 35 (Preussen × MacLaren's Prolific) tinged and green. (The three parents of 34 and 35 are all tinged.)

As regards Crane and Lawrence's colour factors, the parents of these various families would therefore seem to be as follows:

Semper Fidelis A		PPTT
Hornet A		PpTT
Mitchell's Seedling		PpTt
Baumforth A		ppTt
Red Cross		
North Ward		
Preussen		
MacLaren's Prolific		
3/72 or 6/23, or both	(Family 22)	PPTT
6/86 or 3/10, not both	(Family 23)	PPTT
23/50	(Family 33)	PpTT

Deficiency of Chlorophyll.

Crane and Lawrence mention, but do not discuss in detail, a form of chlorophyll deficiency met with very frequently in the present work. Plants entirely without chlorophyll have never here reached a size large enough for potting. But there are also many plants somewhat deficient in chlorophyll ; the deficiency appears to be of at least two types. The common type has occurred in many families in relatively large numbers ; some of the plants are weak, but most appear able to persist indefinitely, and in some cases bear good crops of good fruit. Only one such plant, a yellow fruited individual of Family 3 (Pyne's Royal selfed) has been propagated ; after propagation it deteriorated rapidly in fruit quality, and somewhat in vigour, and soon became worthless even for garden purposes.

The available data are not full enough to show the inheritance of this "long-lived" type of chlorophyll deficiency. It occurs most commonly in association with green spines ; the exact numerical relationship is, however, difficult to observe. Amongst green spined plants, those with no deficiency stand out conspicuously on account of their dark green foliage ; amongst red and tinged spined plants the distinction seems less clear cut, and is sometimes doubtful.

In Family 6 (Lloyd George selfed), amongst 91 red spined plants (none were tinged), 9 were noted as deficient in chlorophyll. Of 17 green spined plants, 14 were clearly deficient ; only 3 had outstanding dark green foliage. Similarly, in Family 21 (Pyne's Royal × Lloyd George), out of 128 red spined plants only 2 were noted as deficient ; whilst of 39 green spined plants, 30 were clearly deficient.

In Families 22 and 23 (Seedlings crossed) no deficient plants were observed.

In the later families, the green spined plants were discarded too early for observation ; but a few chlorophyll deficient plants amongst the remainder were noted in Family 32 (Preussen × Lloyd George). A total of 262 red spined plants (none were tinged) recorded included 8 noticeably deficient.

Observation of the plant and fruit character of deficient plants with red fruit does not suggest any clear association. Thus of the 9 plants in Family 6 (Lloyd George selfed), 1 was very weak and 1 weak ; the remainder were medium or above in vigour. Of the 8 plants in Family 32 (Preussen × Lloyd George), 2 were weak and 1 rather weak ; 5 were medium. Of fruit characters, only soft fruit seems at all likely to be associated with the deficiency, and even here the association is not very close. In neither brightness nor depth of fruit colour was there any suggestion of association.

A second and completely distinct type of chlorophyll deficiency occurred in Family 17 (North Ward selfed). Of 119 plants observed, 100 had tinged spines and 19 green ; 3 green spined and no tinged plants showed the type of deficiency described above. In the spring of the second year, of 117 plants surviving, 20 were recorded as having "no chlorophyll" (although this was not recorded in the previous summer), and the 3 mentioned above as being deficient. Seventeen of the 20 had tinged spines, and 3 green. Ten of the 20, including all 3 with green spines, died early in the second year. The remaining 10 survived two years more; in every case, the plants appeared each spring with foliage whitish or yellow, seeming to have no trace of chlorophyll; each summer they gradually developed enough chlorophyll to keep the roots alive during the succeeding winter. The canes never reached as much as a foot in height; they died to the ground each winter, except in one case, where a plant produced two small fruits.

The writer has been informed by Mr. R. V. Harris that a form similar to this has been found amongst raspberry seedlings in Canada, and has been regarded as a possible new form of virus disease. Both this and the form of deficiency previously mentioned are here almost certainly of genetic origin. Both are also quite distinct from the type of chlorosis commonly seen in the variety Devon, and known as "Devon chlorosis"; this may or may not be a pathological condition.

Inheritance of Sex.

Crane and Lawrence show that, as regards sex, raspberry plants are of four types, hermaphrodite, female, male and neuter. Female sterile (i.e. male and neuter) plants have characteristic foliage of the type known botanically as Rubus Idaeus obtusifolius. In the present work, the number of plants with foliage of this type has been recorded, but no attempt has been made either to record female plants, to distinguish between male and neuter forms, or to investigate the sex constitution of the parents.

Eight out of nineteen named varieties have, when selfed, produced female sterile plants; these are Baumforth Seedling B, Black Antwerp A, Hornet A, Mitchell's Seedling, Norwich Wonder A (and syns. Fillbasket A, Storrie's Invincible), Red Cross, Superlative A and Winkler's Seedling. Doubtless there are other named varieties in cultivation which will also give female sterile plants; it is probable at least that the following ten will not: Bath's Perfection, Baumforth A, Burnett Holm, Lloyd George, Northumberland Fillbasket Thornless, North Ward, Preussen, Pyne's Royal, Red Antwerp A and Semper Fidelis A. The thirteen plants of Family 24, Owasco selfed, do not provide a safe conclusion.

Since all the eight varieties here shown to have given female sterile plants have been more or less grown commercially, and since under field conditions self-sown seedlings are very common, it is natural to ask why sterile forms are not more often met with. The answer is that until the bulk of these varieties were supplanted by Lloyd George they *were* fairly common. Sterile "rogues" have been seen by the present writer in mixed plantations in which part of the plants were of the following varieties: Scottish "Antwerps" (mainly Black Antwerp A and Red Antwerp A mixed), Hornet A, Mitchell's Seedling, Norwich Wonder A, Steele's Victoria (? syn. Norwich Wonder A), Superlative A.

The case of Superlative A was particularly interesting. Beds of raspberries, alleged to be Superlative, had been planted over a series of years in a large garden in Perthshire. The gardener was much worried because, no matter how heavily he fed his raspberries, the bulk of them would not fruit. The writer found they consisted of perhaps one-tenth Superlative A, and nine-tenths a sterile "rogue". The "rogue" was so much more vigorous than Superlative that it had taken nearly all the space, helped, of course, by the fact that it provided the best "spawn" for planting fresh beds.

There is also the curious case of Red Antwerp B, a variety formerly often found as a "rogue" in Scottish plantations (chiefly of "Antwerps" and Norwich Wonder A), and sometimes in England; the same variety was also received at East Malling free from mixture, as "Red Antwerp" and "Carter's Prolific". Though this variety has no commercial value, it was found useful at East Malling at the beginning of investigations into raspberry diseases. In order to get canes free from disease, self pollinated seedlings of several varieties were grown; the writer three times tried to self pollinate the flowers of Red Antwerp B (not then knowing that female forms existed), but could never find any well developed anthers, much less any pollen. Not a single self pollinated fruit was ever obtained; the variety is presumably a female "rogue", complementary to the male sterile "rogues" just mentioned.

The opinion hazarded by Crane and Lawrence that probably "the heterozygous sex condition is correlative with convex and curved foliage", such as that of Superlative A and Norwich Wonder A, is not fully borne out. Though none of the varieties yet known to give sterile forms is as flat-leaved as, for instance, Baumforth A, at least three, Hornet A, Mitchell's Seedling and Red Cross, have leaves not at all strongly curled, except when they are growing in very dry conditions; and one variety, Pyne's Royal, which does not give sterile plants, has foliage quite as strongly curled as that of Superlative, which it resembles, and more so than that of Norwich Wonder A.

Inheritance of Hairiness.

Crane and Lawrence show that varieties with "subglabrous" canes, when selfed, produce only subglabrous plants. The three hairy-caned forms used by them in selfing and in crossing amongst themselves, gave more nearly a 2 to 1 than the expected 3 to 1 ratio of hairy to subglabrous plants; and none proved to be homozygous for hairiness. These points are confirmed, at least in part, by the present work. Subglabrous varieties when selfed or crossed amongst themselves have never given any approach to the hairy type, though several of their seedlings have been definitely more hairy than the varieties themselves. Of the four large families raised by selfing hairy caned varieties, none was free from subglabrous forms; three, Lloyd George, Norwich Wonder A and Red Cross, with 79 hairy and 28 subglabrous, 98 and 37, 40 and 17 respectively, have some excess of subglabrous plants. The fourth, Northumberland Fillbasket Thornless (Family 15) gave 81 hairy and 6 subglabrous (expectation 65·25 hairy, 21·75 subglabrous), a large excess of hairy. The four families together give a reasonable approach to expectation, 298 hairy to 88 subglabrous, instead of 289·5 to 96·5.

Four families were produced by crossing hairy and subglabrous forms. Two of these, Families 21 and 32 (Pyne's Royal, and Preussen × Lloyd George respectively) each gave an excess of subglabrous forms; in the former the excess was considerable, 68 hairy to 97 subglabrous, where equality was expected. Families 22 and 23, each raised by crossing a hairy with a subglabrous seedling, gave in each case a small excess of hairy plants. The four families together again give an approximation to the expected equality, with 308 hairy to 330 subglabrous.

The fact that in addition to the three hairy forms tested at Merton, four more forms tested at East Malling all prove to be heterozygous for hairiness greatly strengthens Crane and Lawrence's suggestion that the homozygous forms may be suppressed by a lethal factor. The only difficulty is Family 15 in the present work, with its large excess of hairy plants. We have to consider also the facts that while at least in the writer's experience, amongst wild raspberries far from cultivation, the hairy type predominates, in the named varieties now grown at East Malling (even excluding the American varieties, which are all subglabrous), subglabrous varieties predominate by almost four to one. The explanation of these facts is not apparent.

Absence of Spines.

The variety, "Northumberland Fillbasket Thornless", which was selfed to produce Family 15, is not absolutely free from spines. A very few long, tinged spines, usually bright red at the tip, can sometimes be found close to the ground on young canes; and the backs of the leaf midribs frequently carry a few similar spines. In fewness of spines the selfed family closely resembles its parent, yet on close examination none could be classed as entirely free from spines, though none had more than a few, and these close to the ground or on the leaf midribs.

In Family 25 (Burnett Holm selfed), there appeared for the first time in the present work plants which, on the closest examination, proved to be entirely free from spines on every part—the botanist's *forma inermis* ; they comprised ten plants out of thirty-six, a close approach to the 3 : 1 ratio. The family was much too small, and grew too badly to show any possible numerical relations between this and other cane and fruit characters ; it was observed, however, that the ten spine-free plants included forms with hairy and subglabrous canes, and also both red and yellow fruited forms.

If, as seems probable, this spine-free character acts as a simple recessive, besides its genetic interest, it may be of use to the breeder. We have already seen that comparative freedom from spines is commercially desirable ; since many seedlings in nearly all families have proved to be rather spiny, an F_2 generation from crosses between the unarmed type and any good variety or seedling should at least provide more promising material as parents. Nearly all the seedlings of Family 25 already carry the "pale" fruit character ; and most are "firm" ; thus these three characters should appear together in at least a small proportion of the F_2 seedlings.

Abnormal Types of Inflorescence and Flowers.

The normal inflorescence of Rubus Idaeus is a terminal raceme, usually with one or more secondary racemes in the axils of the leaves towards the base of the flowering shoot. In Family 16 (Red Cross selfed) appeared a few plants with a somewhat flat-topped inflorescence resembling a corymb. Owing to the poor growth of some plants through what appeared to be partial winter killing of the canes, their number could not be accurately determined. Amongst four plants in which the type was well developed, one had green spines and yellow fruit ; all were fertile, none male or neuter. These plants bore abundant and well developed fruit, quite up to the average of the family as a whole.

The present writer cannot remember having previously seen a red raspberry, either European or American, with this type of inflorescence. The inflorescence of the American black raspberry, Rubus occidentalis, is similar but more truly "corymbose" than the type here referred to. Many of the "purple cane" raspberries, hybrids between red and black varieties, also have an inflorescence of this type. The writer is indebted to Mr. Pyne, the raiser of Red Cross, for the information that, although the parentage of Red Cross is unknown, it is probable that plants of Shaffer's Colossal, a "purple cane" variety, were growing in the plantation from which the seed or seedling was obtained. It is thus at least possible that the appearance in Red Cross selfed of some plants with a corymbose inflorescence is due to a trace of R. occidentalis in its parentage.

In Family 25, Burnett Holm selfed, out of twenty plants which eventually flowered, three, all yellow fruited, produced peculiar flowers, most of whose petals contained chlorophyll, and resembled the sepals. A few flowers on each plant had one or more petaloid stamens. In other respects the flowers were normal ; they developed fruits of moderate quality. So far as the writer has observed, this type of flower has not appeared in any other family ; he has not observed anything similar amongst wild or cultivated raspberries anywhere. The genus Rubus, however, contains a number of horticultural forms with "double" flowers, a parallel variation.

A Seedling Botanically Identical with a Named Variety.

The variety Red Antwerp B, with female flowers, has already been mentioned as a "rogue" commonly found in Scottish plantations of Norwich Wonder A, and other varieties. In Family 10 (Norwich Wonder A selfed), a plant was found which appeared in all respects to be identical with Red Antwerp B. It was propagated and planted alongside plants of the latter variety for close comparison ; the only difference noted over several seasons was a slightly greater vigour and more profuse "spawn" production of the seedling. Since both plants appear to be female, nothing short of appropriate crosses would show whether the two are genetically identical ; this has not been thought worth while. This is the only instance known to the writer in which forms botanically indistinguishable have arisen as different seedlings amongst fruits as heterozygous as are most raspberry varieties, and particularly Norwich Wonder A.

In conclusion, the writer would like to express his sincere thanks to Mr. Richard Wellington and Mr. E. G. Ing, for their help and advice in describing the fruits of many seedlings.

SUMMARY.

An account is here given of observations made over a period of thirteen years on thirty-five families of raspberry seedlings, produced by selfing and crossing named varieties and seedlings.

The objects of the work ; the characters of a good commercial raspberry ; varieties at present in cultivation from which the breeder might obtain these characters ; and a score card for judging the commercial value of the fruit, are in turn described. The early growth and fruit bearing of raspberry seedlings are discussed in relation to juvenility.

An attempt is made to show the comparative value as parents, both when selfed and when crossed amongst themselves, of different named varieties and seedlings. The most valuable commercial varieties do not necessarily produce, when selfed, the largest proportion of promising seedlings ; nor do those varieties which, when selfed, give the largest proportion of promising seedlings, always give equally promising results in crossing.

The most hopeful line of work so far found, and applicable probably outside the limits of raspberry breeding, is the crossing of promising seedlings belonging to different families, and particularly to different selfed families. It is important that selfed seedlings chosen for crossing should not have the faults of their parents in a degree equal to that of the parents.

Though the work was not primarily designed to give results of genetic interest, the bearing of the available records on the published results of Crane and Lawrence in the inheritance of colour, sex and hairiness, is discussed. Some additional characters of mainly genetic interest are described.

REFERENCES.

(1) *Hedrick, U. P.* and *others.* The Small Fruits of New York. Albany, N.Y., 1925.

(2) *Crane, M. B.* Studies in Relation to Sterility in Plums, Cherries, Apples and Raspberries. Memoirs of Hort. Soc. of N.Y., 3, July 1927.

(3) *Crane, M. B.* and *Lawrence, W. J. C.* Inheritance of Sex, Colour and Hairiness in the Raspberry, Rubus Idaeus L. Journ. of Genetics, 1931, 24, 2.

(4) *Grubb, N. H.* and *Peren, G. S.* A Note on the Impurity of Raspberry Stocks. Journ. of Pom. & Hort. Sci., 1922, 3.

(5) *Grubb, N. H.* Commercial Raspberries and Their Classification. Journ. of Pom. & Hort. Sci., 1922, 3, 1.

(6) *Harris, R. V.* and *Grubb, N. H.* The Commercial Control of Raspberry Mosaic Disease. East Malling Res. Sta. Ann. Rpt. 1932.

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A NOTE ON THE RECOVERY FROM SILVER-LEAF DISEASE OF PLUM TREES ON COMMON PLUM AND MYROBOLAN STOCKS RESPECTIVELY

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Some years ago it was noted by one of us (Brooks and Storey, "Silver-leaf Disease—IV", Journ. of Pom. & Hort. Sci., **3**, p. 4, 1923) that in an East Anglian fruit plantation Victoria plums, then about twenty-two years old, worked on Common Plum stock, were much less affected by Silver-leaf disease than those on Myrobolan stocks. The difference in incidence of the disease on the two classes of trees in this plantation is also mentioned by Petherbridge (Journ. of Pom. & Hort. Sci., **5**, p. 141, 1926). The trees on Common Plum had been worked at a height of two or three feet whereas those on Myrobolan had been worked in the usual way. There was a striking difference in the habit of the trees on the two stocks, with which perhaps the difference in incidence of Silver-leaf disease was associated. The belief in the resistance to this disease of Victorias worked on Common Plum is referred to by Hatton, Amos and Grubb (East Malling Res. Sta. Ann. Rpt. 1924, p. 97).

On mentioning the above observation to Mr. R. G. Hatton, he kindly arranged that a plantation of Victoria plums, worked on Common Plum and Myrobolan B, should be established at East Malling, some being budded or grafted at the usual level, others being budded or grafted high up on the stock, with a view to seeing whether the observation made in the East Anglian fruit plantation could be confirmed. The times of budding and grafting, either in the usual manner or high up on the stock, were chosen so that the trees were at a comparable stage of development at the time the observations began. The trees were planted in contiguous rows and were pruned in the usual manner.

To have observed the natural incidence of Silver-leaf disease in these trees would have entailed the maintenance of the experiment for many years. It was thought, however, that interesting data might be obtained over a shorter period of time by inoculating the trees with *Stereum purpureum* and by determining the rate of recovery from attack in the different classes; the latter would probably be some measure of the degree of resistance to Silver-leaf disease.

In March 1930, when the trees were about five years old, three of the larger shoots on each tree were inoculated with a uniform spore-suspension of

Stereum purpureum. All these shoots were silvered to approximately the same degree in June 1930. Records of the behaviour of the trees were taken each summer. The trees were not manured in any way. In some trees the disease spread to such an extent that they began to die back and had to be removed, in others there was complete recovery from the disease. Unfortunately several trees were killed by Bacterial Canker during the early part of the experiment, thereby seriously reducing the number of trees under observation. In consequence, no conclusions can be drawn as to the relative merits of working the trees "high" or "low". By August 1934, however, there was a striking difference in the amount of recovery from Silver-leaf disease of the trees worked on Common Plum and Myrobolan B respectively. The position then was as follows:

TREES ON MYROBOLAN B (13)

Grafted high (4)

Killed by Silver-leaf	0
Silvered	4
Healthy	0

Grafted low (1)

Killed by Silver-leaf	1

Budded high (3)

Killed by Silver-leaf	0
Silvered	2
Healthy	1

Budded low (5)

Killed by Silver-leaf	3
Silvered	1
Healthy	1

TREES ON COMMON PLUM (15)

Grafted high (2)

Killed by Silver-leaf	0
Silvered	1
Healthy	1

Grafted low (2)

Killed by Silver-leaf	0
Silvered	1
Healthy	1

Budded high (5)

Killed by Silver-leaf	0
Silvered	0
Healthy	5

Budded low (6)

Killed by Silver-leaf	0
Silvered	1
Healthy	5

In summary form the results are:

TREES ON MYROBOLAN B (13)

Killed by Silver-leaf	4
Silvered	7
Healthy	2

TREES ON COMMON PLUM (15)

Killed by Silver-leaf	0
Silvered	3
Healthy	12

The high rate of recovery of the trees on Common Plum is probably related to the hot, dry summers of 1933 and 1934.

Although the number of trees which remained in the experiment is small, the difference in the course of the disease in the trees on Common Plum and Myrobolan B respectively, may be significant. It has been thought worth while to publish this note as it is only by the accumulation of data of this kind that any confident statement can be made ultimately about the influence of the stock on the incidence of Silver-leaf disease in plum trees.

In addition to the above results, Mr. Hatton has kindly called our attention to some data available from a Plum Stock Trial at the East Malling Research Station, which also appear to indicate a greater measure of recovery from Silver-leaf disease in trees on Common Plum stock than in those on Myrobolan (seedling stocks).

These data concern the natural infection of plum trees in Plot 20 by Silver-leaf disease and their subsequent recovery. We are greatly indebted to the late Miss A. D. Mackenzie for these records and to Mr. T. N. Hoblyn for their evaluation.

Plot 20 was the first plum stock trial including more than one variety to be planted at the Station, the trees being planted as maiden trees in February 1921. Ten varieties were included and nine rootstocks, but as in those early

Plot 20. Plum Rootstock Trial. Effect of Rootstock on Recovery of Trees from Silver-Leaf.

Varieties on	COMMON PLUM.						MYROBOLAN.					
	No. of trees planted 1921.	Never silvered.	Silvered.	Killed by Silver-Leaf.	Still silvered 1931.	Recovered by 1931.	No. of trees planted 1921.	Never silvered.	Silvered.	Killed by Silver-Leaf.	Still silvered 1931.	Recovered by 1931.
Victoria	8	0	8	1	2	5	8	1	7	3	2	2
Czar	3	1	2	—	—	2	17	1	16	1	4	11
Pershore Egg ..	7	3	4	—	—	4	8	—	8	1	—	7
Denniston's Gage ..	6	3	3	—	—	3	8	4	4	—	1	3
Belle de Louvain ..	8	2	6	—	—	6	8	2	6	1	2	3
Total	32	9	23	1	2	20	49	8	41	6	9	26

Silvering.

Totals.	Common Plum.	Myrobolan.	
Not silvered ..	9	8	17
Silvered ..	23	41	64
	32	49	81

There is no significant difference in numbers affected.

Recovery.

Totals.	Common Plum.	Myrobolan.	
Not recovered	3	15	18
Recovered ..	20	26	46
	23	41	64

A significantly greater proportion of the trees on Common Plum has recovered. (P is between ·05 and ·02.)

years the number available of certain rootstocks was small, all combinations of stock and scion were not included and in some cases very few trees were available for planting.

During 1922 the plantation was severely attacked by Silver-leaf disease, 249 trees out of 462 being affected in this year. Twenty-four more trees were attacked in 1923, but after that date there were comparatively few new cases, the total number of trees attacked up to 1931 being 284.

By 1923, 108 of the trees originally affected had recovered and although some of these became re-affected subsequently, the majority of the trees had recovered by 1931. Actually, of the 284 trees silvered during the ten year period, 206 recovered. All the plums were grubbed in the winter, 1932-33, to make room for the pears interplanted with them.

During the period a number of trees were attacked by other diseases and pests, but no account is taken of them in the following Table.

This Table compares the attack and recovery of trees on Myrobolan (seedlings) and Common Plum stocks ; and the five varieties which include both stocks are shown. It should be noted, however, that there are only three trees of Czar on Common Plum.

There was no significant difference in the number attacked on the two stocks, but the proportion of recoveries on Common Plum seems to be just significantly higher than on Myrobolan.

This significance is reduced if the Czars are omitted, and without other evidence could not be taken too seriously. In view, however, of the other results recorded above, the higher proportion of recoveries on Common Plum than on Myrobolan is of great interest.

(*Received* 26/3/35.)

THE FUNGUS FLORA OF APPLE TWIGS AND BRANCHES AND ITS RELATION TO APPLE FRUIT SPOTS

I. REVIEW OF LITERATURE AND PRELIMINARY EXPERIMENTS

By L. OGILVIE
Long Ashton Research Station

It is well known that several of the most important fungi responsible for fruit spotting of apples also occur commonly on the twigs and branches. Those which cause noticeable cankers have been specially studied. In several cases fungi which are known to cause bark cankers in other countries have been isolated from fruit spots here, but in this country their association with cankers has not been proved. It is also insufficiently realized perhaps that dead twigs, pruning snags, etc., are a very favourable culture medium for certain of the less common fruit spotting fungi.*

The object of the present paper is to draw attention briefly to those cases in which the connection between twig or branch and fruit infection is well established and to give a short account of some observations and experiments bearing on the second point.

A. Fungi Parasitic on Living Twigs and Branches.

It has long been known that the apple scab fungus (*Venturia inaequalis*) can infect green twigs. The fungus gains entrance near the tips where bark formation has not taken place and forms a pad of tissue just under the cuticle during the summer growth period. The history of the infection cushion has been followed by Marsh and Walker (21), who have shown that subsidiary pustules are formed during the winter, these being the only portions of the fungus tissue of importance in spore production. Spores are produced from them during the whole period of opening of the blossom buds.

Sclerotinia fructigena, the cause of brown rot of apples, sometimes extends along the stalk of affected fruits and penetrates the wood of certain soft wooded varieties, such as Lord Derby and James Grieve, and produces cankers at the bases of the spurs (34). The fungus may overwinter in this way and infect fruits in the following year, but mummified fruits appear to be the usual source of this infection.

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* Since this paper was written the following interesting paper, dealing mainly with plum and peach, has come to hand: Koch, L. W., "Studies on the Over-wintering of Certain Fungi Parasitic and Saprophytic on Fruit Trees," *Canad. Journ. Res.*, II., pp. 190-206, 1934.

The common canker fungus, *Nectria galligena,* frequently infects the fruit also, as has been shown by Dillon Weston (7), the fungus entering at the calyx end and then producing a rather rapid decay. It has also been observed on shrivelled fruits which have remained on the trees throughout the winter (8). In some seasons considerable injury is caused to fruit. Thus, in 1932 affected apples were sent in from many parts of the Bristol Province, Worcester Pearmain being the variety mainly attacked.

Glomerella cingulata (*Gloeosporium fructigenum*), the cause of "bitter rot" of apples, is often destructive in the United States. In this country, although often confused with *Gloeosporium album*, which will be referred to later, it undoubtedly causes a good deal of injury. Thus in 1933, it was very common in store on samples of Cox's from Essex and of Bismarck from Worcestershire. On both these varieties the lesions are circular, light brown, and bear concentric rings of minute acervuli.

It has been shown by Spaulding and Von Shrenck (33) in the United States that the fungus can form short lived cankers on the branches, and there is a questionable record of their occurrence in this country in a paper written by M. C. Cooke in 1903 (4). The cankers are usually elliptical, dark in colour, slightly sunken and adhering to the wood and often zoned. Mummified fruits are also instrumental in the overwintering of the fungus.

Phacidiella discolor, previously recorded from Russia, was found by Nattrass causing canker and die-back of head-worked trees in Herefordshire in 1926 (26). It has been recorded also from Cambridgeshire (2) and from Birmingham. A rot of apples in storage, in which the skin becomes black and the flesh brown to brownish black, caused by the same fungus, has been described by Osterwalder (29) from Germany and by E. and E. Marchal (18) from Belgium.

Fusarium lateritium var. *fructigenum* causes a fairly commonly occurring spotting and rotting of apples and is also responsible for a bud rot which is prevalent in the Eastern Counties.

Physalospora Cydoniae (*Sphaeropsis Malorum*) is occasionally found on apple fruits in England, causing the disease known as "black rot". The spots are at first brown but as they enlarge they darken and finally become black. "Later stages in the development of the rot show a shrivelled and much wrinkled surface, which typically becomes covered with black pustules. Ultimately a dry mummy is produced, which may hang on the tree for a year or more" (12).

The fungus also attacks twigs and branches, causing reddish brown cankers. These become sunken and may cease growing at the end of one season, or may extend year after year so that large cankers with concentric crevices are produced. Some injury is usually to be found on branches or twigs where the fungus has gained entrance.

On the leaves characteristic "frog-eye" spots are produced, but these seldom bear fruiting pustules (5). It is possible that this leaf-spot may occur in England (24).

The fungus occurs in most apple growing regions of the world. Hesler and Whetzel (13) state that "speaking generally for north-eastern America, the Middle West and south-eastern Canada, this apple disease in one or more of its three forms stands second only to apple scab in importance". It appears to be only of minor importance, however, in European countries.

The fruit rot was first recorded from England by Berkeley in 1836, and since then has been occasionally reported. Kidd and Beaumont (17) found it only rarely in store although, according to them, Horne had recorded it as a common rot-producing fungus. The canker stage does not seem to have been reported from England.

Neofabraea malicorticis (*Gloeosporium malicorticis*) is the cause of "anthracnose", a serious disease of apple trees in British Columbia and Oregon. On the fruits a slowly progressing "bull's-eye" type of rot is produced. The fungus also infects the twigs and branches through uninjured bark. The cankers are elongated and show transverse slits or cracks, through which the acervuli appear. They remain active only for two seasons (10).

A closely similar disease of apple trees occurring in Oregon and Washington, and also in British Columbia and New Zealand, is caused by *Gloeosporium perennans*. Prevalence of this disease appears to be closely bound up with low winter temperatures and invasion of the tissues by woolly aphis (36). The fruit spotting is also of the bull's-eye type and has been well described by Heald (10). Neither of these fungi is known to occur in England, although the latter much resembles *G. album*.

Apple "blotch" (9, 31) is another serious American fruit spot which is at present unknown in England. The fungus, *Phyllosticta solitaria*, causes conspicuous star-shaped lesions on the fruit. Very small white spots are also produced on the leaves and small cankers on the twigs, which eventually become tan-coloured. These cankers may slough off, resulting in large roughened areas on the twigs and small branches.

B. FUNGI SAPROPHYTIC ON DEAD TWIGS AND BRANCHES.

We now have to deal with certain fungi which have been found on dead apple twigs and which can also infect apple fruits.

Gloeosporium album is one of the commonest "lenticel-spot" fungi of apple fruits in the Bristol Province, and is associated with much damage in the later stages of their storage life. It has been recorded previously by Kidd and Beaumont (17). At Long Ashton it is especially common on Allington Pippin. The spots are circular, flat or concave and chestnut brown, with concentric rings of pale acervuli, and spread slowly till the whole of the fruit may be mummified.

The disease has been studied for several years by the writer. It was found, in 1933, that at the same time as the apples are ripening, spores are being produced from old mummified fruits and from small cankers on pruning snags. No very successful results have followed inoculation of pure cultures on living twigs and it is at present thought that the fungus can only infect dead twigs or progress from them very slightly into healthy wood.

During 1933 apples were received from very many sources which were affected with a characteristic black rot. The rot commenced as sunken dark brown areas which developed in the centres of lenticel spots. These later enlarged slowly to ill-defined chestnut, brownish black to black areas which finally involved the whole of the fruit, which became black and mummified. This rot was eventually found to be due to an *Alternaria sp*. Inoculations of injured apple fruits with pure cultures produced in every case the typical rot, but it was not possible to infect uninjured fruits.

In February 1934, a large number of inoculations were made with pure cultures on healthy twigs and on twigs which had previously been burned. No result was apparent by the end of September in the case of the normal twigs but in the case of the burned ones the fungus had formed, in every case, large cushions which were sporing profusely. It was also abundant in the galleries of larvae of the beetle, *Tetrops praeusta* L. which had penetrated the twigs. Examination of dead twigs from other sources revealed that the fungus is a very common saprophyte on such twigs.

Several species of *Alternaria* have been recorded from apple fruits. In this country Horne (14) has recorded *A. Grossulariae, A. pomicola* and *A. tenuis*. Kidd and Beaumont (17), found *A. tenuis* and showed it to be capable of infecting the apple through the lenticels but only if these were first injured by ammonia vapour. It has also been found to occur commonly on apples in Washington State in the United States (11). In that country *Alternaria* rots frequently follow lesions produced by Jonathan Spot and Bitter Pit. *A. Mali,* besides causing a fairly rapid rot of apple fruits, can bring about enlargements of dead spots on apple foliage (30). It has been found here, as in the United States, that *Alternarias* the fungus frequently invade the interior of fruits with open eyes or accompany injury to the fruits by boring larvae, such as those of codling moth.

Many of the burned areas under observation in connection with inoculations of fruit spot fungi on twigs also developed abundant cushions of *Botrytis cinerea* under the bark. This fungus is of course a very common cause of rotting of apple fruits. The usual colour of the lesions is yellowish grey while the characteristic feature of the rot is the soft bladder-like appearance of the affected area. In its final stages the interior of the apple becomes water soaked and has an aromatic odour. In "Fungus and other Allied Diseases of Crops, 1928-32", (23), issued by the Ministry of Agriculture and Fisheries, it is stated that "in Yorkshire in 1932, *Botrytis* sp. was associated with and apparently responsible for cankers on shoots of Allington Pippin grafted in the previous year". *B. cinerea* was noted on roots of laid in apple stocks at East Malling in 1932 by Wormald (35).

One of the commonest fungi occurring on dead apple twigs is *Diaporthe perniciosa*. There has been much controversy concerning the parasitic rôle of this fungus. It is associated with diebacks of apple trees, as has been shown by Briton Jones (1) and others. Inoculations of the fungus by the author on healthy twigs have been unsuccessful and the fungus has not progressed from burned to healthy areas. It is therefore considered by the writer, as by Briton Jones, to be mainly a saprophyte. It has not been found on fruit at Long Ashton, but E. and E. Marchal (18) in Belgium and Kidd and Beaumont (17) in England have isolated it frequently from apples. The latter state "When apples are stored for any considerable period the importance of the fungus is second only to that of *Penicillium expansum*, but being essentially a storage rot, it is relatively unimportant during the short period apples are normally kept." The rot is at first smooth, rather dark brown, often lighter at the margin. It progresses steadily towards the centre until the apple has become brown throughout. The surface remains smooth but eventually there appear from under the skin masses of closely packed, small, black stromatic bodies. Positive inoculations have been obtained at Long Ashton with cultures isolated from twigs.

Perhaps the commonest saprophyte occurring on dead apple twigs, often in conjunction with *Diaporthe perniciosa*, is *Valsa ambiens*, of which the imperfect stage is *Cytospora ambiens* (27). Twenty-four inoculations with pure cultures of this fungus on apple fruits have, however, been unsuccessful. *Myxosporium corticola*, the cause of "superficial bark canker" and another somewhat less common, weakly parasitic fungus, is also apparently harmless to the fruits.

A fungus resembling *Coniothecium chomatosporum* occurs very commonly on both living and dead apple twigs in England. Cunningham (6) states that the *Coniothecium* produces small blisters on the bark of twigs and branches of apple trees in New Zealand, and also marked russeting of the fruit. Moore has worked on this and other similar fungi in England, but in a Progress Report (25), states that pathogenicity has not yet been proved by inoculation.

ORCHARD PROBLEMS RELATING TO FRUIT ROTS.

From the literature and observations recorded above it will be clear that much of the infection of apple fruits may be traced to the twigs and branches of the tree. The majority of cankers on apple trees occurring in this country are due to the common canker fungus, *Nectria galligena*, but in the writer's opinion a critical investigation of apple cankers occurring here is urgently required.

Horne and others (16) and recently Miss F. M. Carter (3) have studied the distribution of spores in the air of orchards. Horne and his co-workers found that spores of *Fusicladium* predominate, especially in early summer, that *Botrytis* is abundant, especially in late autumn, while *Alternaria, Pleospora* and *Phoma* are moderately abundant. Miss Carter identified some forty-five genera of fungi obtained from the exposed plates, among them, in addition to the above, being *Fusarium fructigenum* and *Trichothecium roseum*.

These workers have found that most of the fungi caught by them on plates exposed above orchards are known to occur on apples, and conversely, that the majority of the fungi isolated by them from diseased apples at all stages of maturity appear on their lists of fungi caught on plates. Hence apple rots in storage are thought by them to be due, to a great extent, to fungus infection from the air before picking.

Horne's experiments have also shown that orchards which have a reputation for apples of good keeping quality on account of low fungus infection have low atmospheric spore populations.

One must not forget that certain soil inhabiting fungi, as the writer has shown in the case of *Phytophthora Syringae* (28), and fungi occurring on debris may be of some importance in this relation to fruit spotting. Many of the fungi listed by Miss Carter could have arisen in this way. The nature of the orchard floor, the undercropping, the cultivation and the general ochard hygiene are probably of importance in this respect. Horne (15), for example, has found the important rot fungus, *Trichothecium roseum*, on decaying apple leaves and other apple fungi on grasses, and the writer has been struck by the specificity of fungus attack on apples derived from various orchards.

ORCHARD CONTROL MEASURES AGAINST FRUIT SPOTS.

It is the opinion of the writer that modern orchard practices, such as the early removal of dead twigs, branch snags, mummified fruits, etc., and routine sprayings as carried out for the control of apple scab, do much to mitigate fruit infection. In orchards where certain of the fruit spotting fungi become prevalent the branches should be examined for cankers and dead wood and these should be cut out. A special summer application of Bordeaux mixture may be required in the case of varieties not injured by Bordeaux. In the

United States, for instance, such a spray is effective, together with a programme of pruning out cankers, for the control of bitter rot and of blotch. In the case of anthracnose, the control measures advocated in the United States are removal of the cankers and an application of Bordeaux mixture to the trees after the fruit has been harvested. The nature and times of application of the fungicides will, of course, depend on the life history of the fungi concerned.

REFERENCES.

(1) *Briton Jones, H. R.* On the Diseases known as Bark Canker and Die-back in Fruit Trees. Journ. Pom. & Hort. Sci., 4, p. 162, 1925.

(2) *Brooks, F. T.* On the Occurrence of *Phacidiella discolor* in England. Trans. Brit. Myc. Soc., 13, p. 75, 1928.

(3) *Carter, F. M.* A Brief Account of Fungi present in the Air over Orchards, with especial reference to *Pleospora* and *Polyopeus*. Trans. Brit. Myc. Soc., 19, p. 145, 1935.

(4) *Cooke, M. C.* Bitter Rot of Apples (*Gloeosporium fructigenum*). Gard. Chron., 36, p. 249, 1904.

(5) *Crabill, C. H.* The Frog-eye Leaf Spot of Apples. Virginia Agri. Expt. Sta., Bull. 209, 1915.

(6) *Cunningham, G. H.* Fungous Diseases of Fruit Trees. p. 137, 1925.

(7) *Dillon Weston, W. A. R.* Notes on the Canker Fungus (*Nectria galligena*). Trans. Brit. Myc. Soc., 12, p. 5, 1927.

(8) ———. A Preliminary Note on the Perithecia of *Nectria galligena*. Ann. Appl. Biol., 12, p. 398, 1925.

(9) *Guba, E. F.* Phyllosticta Leaf Spot, Fruit Blotch and Canker of the Apple, its Etiology and Control. Univ. of Illinois Agri. Expt. Sta., Bull. 256, 1925.

(10) *Heald, F. D.* Manual of Plant Diseases. p. 500, 1926.

(11) *Heald, F. D.* and *Ruehle, G. D.* The Rots of Washington Apples in Cold Storage. Wash. Agri. Expt. Sta., Bull. 253, p. 13, 1931.

(12) *Hesler, L. R.* Black Rot, Leaf Spot and Canker of Pomaceous Hosts. Cornell Univ. Agri. Expt. Sta., Bull. 379, 1916.

(13) *Hesler, L. R.* and *Whetzel, H. H.* Manual of Fruit Diseases. p. 45, 1922.

(14) *Horne, A. S.* On Spotting in Apples. Gard. Chron. 68, p. 216, 1920.

(15) *Horne, A. S.* and *others*. Biological Work on Fruit. Dept. of Sci. and Indust. Res., Rept. Food Investigation Board, p. 277, 1931.

(16) ———. Pathogenic Fungi present in the Air. Ibid., p. 279, 1932.

(17) *Kidd, M. N.* and *Beaumont, A.* Apple Rot Fungi in Storage. Trans. Brit. Myc. Soc., 10, p. 98, 1924.

(18) *Marchal, El.* and *Em.* Contribution à l'Etude des Champignons Fructicoles de Belgique. Bull. de la Societe Royale de Botanique de Belgique, 44, p. 1, 1921.

(19) *Marsh, R. W.* Spraying Trials against Apple and Pear Scab at Long Ashton. III. Season 1930. Ann. Rpt. Agri. and Hort. Res. Sta., Long Ashton, Bristol, p. 151, 1930.

(20) ———. Apple Scab Control in the Bristol Province. Field Trials 1930. Journ. Pom. & Hort. Sci., 9, p. 53, 1931.

(21) *Marsh, R. W.* and *Walker, Mary M.* The Scab Fungus (*Venturia inaequalis*) on Apple Shoots. Journ. Pom. & Hort. Sci., 10, p. 71, 1932.

(22) *McLarty, H. R.* Perennial Canker of Apple Trees. Canadian Journ. of Res., 8, p. 452, 1933.

(23) *Ministry of Agriculture and Fisheries.* Fungus and other Allied Diseases of Crops 1928-32. Bull. 79, 1934.

(24) ———. Apple Leaf Spot. Journ. Board of Agri., 20, p. 513, 1913.

(25) *Moore, M. H.* Investigations on *Coniothecium.* A Progress Report. East Malling Res. Sta. Ann. Rpt. for 1928-30. II. Supplement, p. 150, 1931.

(26) *Nattrass, R. M.* The Occurrence of *Phacidiella discolor* in the Bristol Province. Ann. Rpt. Agri. and Hort. Res. Sta., Long Ashton, Bristol, p. 99, 1927.

(27) *Ogilvie, L.* Canker and Die-back of Apples associated with *Valsa ambiens.* Journ. Pom. & Hort. Sci., 11, p. 205, 1933.

(28) ———. A Fruit Rot of Apples due to a variety of *Phytophthora Syringae.* Ann. Rept. Agri. and Hort. Res. Sta., Long Ashton, Bristol, p. 147, 1930.

(29) *Osterwalder, A.* *Phacidiella discolor* als Faulnispilz beim Kernobst. Centr. fur Bakt. (2), 52, p. 373, 1921.

(30) *Roberts, J. W.* Experiments with Leaf Spot Fungi. Journ. Agri. Res., 11, p. 57, 1914.

(31) ———. Apple Blotch and its Control. U.S. Dept. Agri. Bull. 534, 1917.

(32) ———. Morphological Characters of *Alternaria Mali.* Journ. Agri. Res., 27, p. 699, 1924.

(33) *Spaulding, P.* and *Von Shrenck, H.* The Bitter Rot of Apples. U.S. Dept. Agri. Bur. Plant Ind., Bull. 44, 1903.

(34) *Wormald, H.* Further Studies of the Brown Rot Fungi. IV. *Sclerotinia fructigena* as the cause of an Apple Canker. Trans. Brit. Myc. Soc., 15, p. 102, 1930.

(35) ——. *Botrytis cinerea* on Apple Roots. Trans. Brit. Myc. Soc., 16, p. 309, 1932.

(36) *Zeller, S. M.* and *Childs, L.* Perennial Canker of Apple Trees. Oregon Agri. Expt. Sta., Bull. 217, 1925.

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NOTES ON THE INHERITANCE OF QUANTITATIVE CHARACTERS IN A CROSS BETWEEN TWO VARIETIES OF GARDEN PEA *(PISUM SATIVUM L.)*

By S. CLAY

PREFACE.

In the year 1927 two varieties of garden pea, "Little Marvel" and a form designated "FB", were crossed together in the hope of obtaining economically desirable types in the hybrid progeny.

It was not until the F_2 (1929) generation from the crosses was being examined in the dry state that the present author became concerned with these plants, and began to attempt some little genetical analysis of the material, alongside the practical plant-breeding. He therefore wishes to record his very great indebtedness to Mr. D. Boyes* for handing over to him the F_2 material, with some notes on the F_1 generation; for his co-operation in the examination of many of the dried F_2 plants, and a large part in the work of labelling the F_3 with dates of first flowering and podding.

Plant breeding, rather than genetical investigation, has continued to be the primary aim of the work, so that the following observations on the mode of inheritance of certain characters are often incomplete.

The partial F_3 (and later generations) have been grown almost entirely on their possible merits as peas, so that they are biassed in many directions, and by no means representative of an unselected F_3 sample.

Data derived from them must therefore be used and interpreted with caution.

The experiment also suffers from the fact that although "FB" is as near to being a pure line as is practically possible, the strain of "LM" used, being of commercial origin, has proved capable of division into a number of lines, differing slightly but significantly in seed size, colour and other features. The genetic purity (for minor details) of the "LM" plants used as parents of these crosses is therefore not assured, and some amount of the variation in the hybrids may be due to this fact. (Note the higher standard deviation of "LM", for seed weight, Table III.) It is therefore particularly unfortunate that the 1929 crop of "LM" grown alongside the hybrid F_2, was not examined and recorded to give exact and comparable data.

It will be seen also, that the number of plants of the other parent, "FB", so analysed, was often too small to be really satisfactory for statistical purposes.

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The work was carried out at the University of Cambridge Horticultural Research Station; and my thanks are due to the Director, Mr. D. Boyes, for granting financial aid to the publication of this paper.

INTRODUCTION.

Five crosses, X.1, 2, 3, 4 and 5, were made using "FB" as female parent and "Little Marvel" (hereafter called "LM") as male. X.6 and X.7 were the reciprocal hybrid. The same two plants were not used as parents in every case.

The F_2 and succeeding generations from X.2 have never shown any trace of "LM" characters, and are therefore in all probability the progeny of accidental selfing; they are of course not included in this paper. The remaining crosses showed no appreciable differences in F_2, with the exception of X.1, which appeared to be abnormal in several respects; it is therefore omitted from the totals in many of the data.

The chief contrasting features of the parent varieties may be summarized as follows:

	"FB"	"LM"
Pod Breadth ..	Broad.	Medium narrow.
Seed Size	Large.	Medium.
Dry Seed Shape ..	Cotyledons flattened.	Cotyledons half-cheese shaped.
Racemes	Few two-flowered.	Many two-flowered.
First Flower ..	Medium early.	Early.
Development of Pod	Fairly rapid.	Rapid.
Foliage Colour ..	Medium green.	Slightly but definitely darker.

In addition, "FB" is rather taller when fully developed, and its first flower is higher up the axis; it branches considerably more than "LM", fills its pods less evenly, more ovules aborting; and it tends to be more susceptible to various diseases, especially to attack by virus "mosaic". These characters fluctuate very widely, and no statistical survey of them has been attempted.

POD BREADTH.

The parent varieties are characterized by pods which in reasonably well-developed specimens, average 18-20 mm. and 15-16½ mm. broad for "FB" and "LM" respectively, this measurement being taken on the ripe dry pod *before* splitting open.

Variation according to environmental conditions, including season and time of year, is very great; all statistics may be very different for the crops of successive years.

Very considerable fluctuation may occur on the individual plant ; ideally the mean of *all* well-developed pods would be obtained as a measure of pod breadth for each plant ; but such a method would be too laborious in practice. Selection of the broadest pod would lead to the inclusion of a number of atypical extremes, especially where faulty development of the contained ovules had led to a failure in the lateral expansion of the pod, a process which normally increases the dorso-ventral measurement appreciably. It was finally decided in this study to utilize the proximal pod (in the case of a two-flowered peduncle) of the second raceme for purposes of measurement, except where some obvious abnormality occurred. This latter reservation unavoidably introduced the personal element to some extent.

The F_2 were quite readily separable by eye into " narrow " (like " LM ") and " broad " (like " FB "). The latter class showed the greatest range, from plants with all pods fully as broad as " FB ", to those with some pods narrow enough to be roughly intermediate between " LM " and " FB ".

The 199 plants scored in this way gave 48 narrow : 151 broad, a very close approximation to the theoretical 1 : 3 ratio of 49·75 : 149·25.

A monohybrid relationship, with broad pod incompletely dominant to narrow, was clearly indicated.

The constitution of " FB " in this respect may be symbolized BB, that of " LM " bb.

Actual measurement tended to obscure this simple relationship.

TABLE I.

No. of Plants.	Pod Breadth (to nearest mm.).	13	14	15	16	17	18	19	20	21	22	23	24
	F_2 (Total 328)	1	4	26	51	38	61	53	69	16	7	0	2
	F_2 from X.I (included in the above) (Total 44) ..		3	1	4	9	10	5	9	2	1		
	" F.B." (Total 36) ..						2	1	17	8	4	4	

(And see Graph I.)

Sufficient figures are not available for a 1929 crop of " LM ", but it is certain that a breadth of 17 was seldom or never reached by pods of that variety.

From the figures for " FB ", grown alongside the hybrids, it will be seen that homozygous broads are unlikely to have contributed to the minimum frequency (but still fairly large) class with a breadth of 17 mm.

This latter was probably composed of heterozygous plants, and was therefore reckoned with the broads. Hence the figures

13-16 mm. (incl.) 82 plants.
17-24 mm. (incl.) 246 plants.

were obtained, an exact agreement with a 3 : 1 ratio!

The centre peak in the distribution curve supports the idea of dominance being incomplete, the heterozygotes showing some tendency to form a class distinct from the homozygous broads, whose mode coincides with that of the curve for the broad parent.

Determination of the constitution of individual plants by examination of the next generation raised from them fully confirm this ; but also indicates that Bb and bb classes were not sharply defined (by measurement) and that some of the 17 mm. group were really " narrow ".

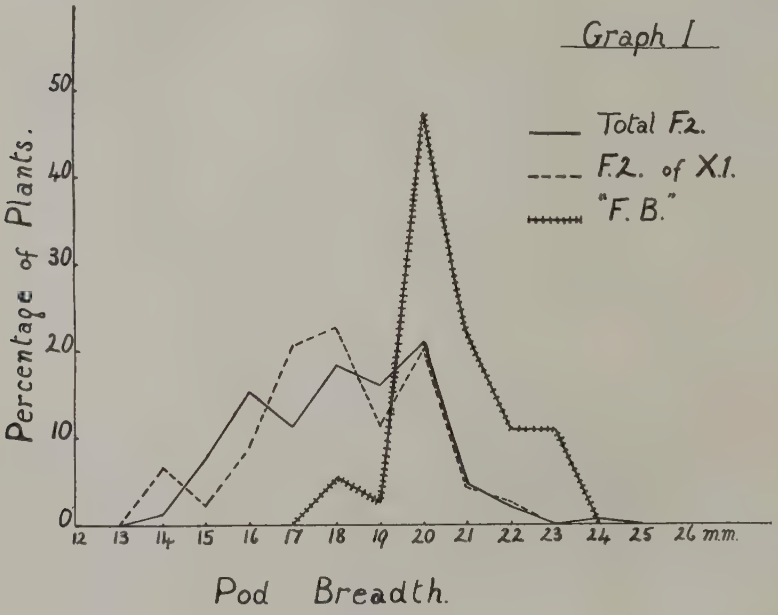


TABLE II.

F₃ behaviour	Pod breadth of F₂ parent.												
	13	14	15	16	17	18	19	20	21	22	23	Mean.	S.E. of Mean.
All broad						2	2	10	1	2	1	20·11 ±1·24	0·29
Segregating ..			1		2	7	3	7	1			18·71 ±1·19	0·28
All narrow ..		1	4	8	2	1						15·87 ±0·93	0·23

Not all the facts are accounted for by this simple scheme. A few plants, more distinctive to the eye than to the millimetre scale, have appeared in quantity in certain F_3 lines, with pods more truly intermediate than those of the heterozygous Bb class. Attempts to investigate their behaviour in succeeding generations have not been very successful, largely owing to the ravages of mosaic, and unusual sensitiveness to environmental influences.

Within the bb class, and less markedly (owing to wider normal fluctuation) within the BB class, various lines have now (in F_3 and later generations) been established with small but persistent differences in mean pod breadth. The best of the bb type has pods as broad as some of the Bb plants.

Broad stipules and leaflets, large flower and possibly thick stem, appear to be determined by the same factor, or one very closely linked to that which produces broad pod. Subsidiary determinants are, however, involved, for at least one line is now, in F_5, characterized by leaflets and stipules definitely rather narrower than those of "FB", although the pods are fully as broad.

Lock (9) crossed "Telegraph" and a "native" pea, with pods respectively about 20 mm. and 13 mm. broad.

The F_2 segregated in the following manner:

Pod breadth ..	19	18	17	16	15	14	13	12
No. of plants ..	1	6	5	8	4	5	2	1

He divided them in the manner indicated to give a 7:17:8 ratio. The end classes so obtained by no means correspond with the parental types, however, and the interpretation offered is unconvincing, especially as no detailed F_3 results are given. In Lock's own words "Extracted recessives seem still to show signs of the influence of the more or less dominant (i.e. broad) form." The above table also indicates that the original large breadth was not recovered.

Lock also found foliage and pod breadth to be associated.

In the cross, "Native" × "Telephone", the broad pod of the latter was dominant in F_1. Lock's classification of the F_2 according to a 3:1 scheme is again unsatisfactory; the parental extremes being ill-represented.

Pod breadth ..	19	18	17	16	15	14	13
No. of plants ..	2	9	22	17	9	14	5
					59	19	

An F_2 from "French Sugar Pea" × "Native", was divided by eye into 65 broad:19 narrow.

Thirteen narrow plants were bred from, 9 proved pure for narrowness, and 4 segregated; of 31 broad plants, 9 proved homozygous and 22 segregated. These results are rather more compatible with a main 3:1 segregation, obscured by overlapping of the classes, somewhat as in the case of "FB" × "LM".

Lock also mentions that the F_1 from "Satisfaction" (22 mm.) × "Native

No. 2" (10 mm.) had a pod breadth of 14-15 mm. If all three figures were taken from the same season's crop, narrowness rather than broadness tended to be dominant here.

Tschermak (20) gives an F_2 segregation of 38 broad podded : 23 narrow pod; and compares it with the theoretical 45·75 : 15·25 (3 : 1)
and 34·31 : 26·69 (9 : 7),
deciding in favour of the 9 : 7 interpretation.

That the chance of the segregation being a monohybrid one is only about 1 in 50, is seen if the standard error, $\pm 3{\cdot}38$, and divergence from theoretical ratio, 7·75, are examined; whereas $\sigma = 3{\cdot}87$ and deviation $= 3{\cdot}69$ on a 9 : 7 basis, supporting Tschermak's decision that two factor pairs were concerned.

It may be mentioned that a form even narrower in pod and foliage than "LM", of the "rogue" type first investigated by Bateson and his co-workers, appeared as a somatic sport in F_2, where one branch on a narrow-podded plant was labelled as being markedly small flowered. Sowing of every pod on the plant separately resulted in a crop of narrow leaved rogues from all the peas borne on the abberrant branch, and all normal plants from the remainder (*cf*. Bateson and Pellew's account (1) of a somatic change in the reverse direction).

A most peculiar plant, stunted, but so excessively branched and rebranched as to form a bush, with very small, almost linear-lanceolate stipules and leaflets, yellow edged, and exhibiting complete sterility, occurred in a badly virus-attacked, broad podded F_4 family.

SEED WEIGHT.

Here, also, variability is very great, and very complex in origin. Unlike, for example, paired flowers (see page 160), seed size is subject to modification by environmental influences acting at practically *any* period of the plant's life history.

A factor of especial importance is attack by the virus disease "pea mosaic". According to time of infection, external conditions, and individual variation in resistance and response to attack, seed may be practically unaffected, more or less reduced in size, or completely aborted. In damp weather, favourable for growth, the mottling of the foliage caused by the disease may be completely masked, but the seed development nevertheless upset. It is therefore frequently uncertain whether the seed of a plant is "normal" in weight, or has been reduced by disease. The crops of successive seasons have been characterized by increasingly widespread and severe virus attack.

On the individual plant great and often inexplicable variations of seed size and shape occur, but these are almost entirely interpod, a high degree of uniformity holding within any individual pod.

The thoroughly dried seed has been weighed ; for convenience the method usually employed being to find the number of peas (discarding those obviously damaged or undersized) just capable of balancing 10 grammes. The numeral so obtained increases inversely as the average weight per seed.

I am indebted to Mr. Boyes for the following figures for the weight of 100 seeds of various batches of "LM" and "FB". In the third column these have been transposed into the form described above.

TABLE III.

Pea.	Weight of 100 seeds. gms.	No. of seeds = 10 gms.	
"FB"			
1	41·0	24·4	Mean = 25·25 ± 1·10
2	40·0	25·0	
3	41·5	24·1	
4	40·0	25·0	
5	36·5	27·4	
6	39·0	25·6	
"LM"			
1	26·5	37·7	Mean = 37·41 ± 2·10
2	26·5	37·7	
3	27·5	36·4	
4	25·5	39·2	
5	28·5	35·1	
6	28·0	35·7	
7	27·0	37·0	
8	28·0	35·7	
9	23·5	42·6	
10	27·0	37·0	

TABLE IV.

Seed of the F_1 plants, grown alongside, where still available, has been weighed.

Plant.		No. of seed = 10 gms. (calculated from weight of 50 seeds).	
	Plant		
X.3	1	26·3	Mean = 27·28 ± 1·06
	2	29·8	
	3	26·8	
X.4	1	26·0	
	3	26·0	
X.5	1	27·8	
	3	27·6	
X.6	2	28·0	
	3	28·7	
	5	26·7	
X.7	2	27·0	
	3	27·4	
	4	26·6	

It should be noted that seed borne on the F_1 plant itself belonged to the F_2 generation. Nevertheless it showed no signs of segregation ; this takes place by *plant*, and has never been demonstrated to occur amongst the seeds of an individual. In other words it is a function of the parent plant, rather than of the seed itself, and the curious anomaly results of some morphological large seeds giving nothing but pure breeding small-seeded progeny.

The standard error of the difference between the means for "FB" and the F_1 hybrids is 0·743. The observed difference, $27·28 - 25·25 = 2·03$, is therefore almost certainly significant of incompleteness of dominance of heavy seed.

The F_2 and the 1929 crop of "FB" were recorded as follows (see Graph 2):

TABLE V.

Seed Weight Index	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	
No. of Plants: F_2		1	6	20	29	38	36	33	29	31	20	16	16	22	16	6	
No. of Plants: F_2 from X.1 (included in above)				2	3	5	4	4	2	4	2	1	2	3	1		
No. of Plants: "FB"					1	2	2	6	8	4	2	1	2				(Mean =27·96)

Seed Weight Index	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
No. of Plants: F_2	5	6	4	6	6	3	1	1	4	3	2				1
No. of Plants: F_2 from X.1	1		1	1				1	1						1

Seed Weight Index	51	52	53	54	55	56	57	58	59	60	
No. of Plants: F_2				1		1		2			(Mean=29·58)
No. of Plants: F_2 from X.1								1			(Mean=30·58)

The figures for "FB" are for comparative purposes misleading, a marked displacement in the direction of small seed being almost certainly a measure of the effect of virus disease too slight to be detected with certainty in the dry state; affected plants had not been marked in the field.

Records for a 1929 crop of "LM" are not available.

It is therefore only possible to compare the F_2 with the parents and F_1 of the previous year.

Fortunately the two seasons were fairly similar in their influence on the respective pea crops.

The general dominance of large seed is emphasized.

A "theoretical" mean of 29·32 calculated from the 1928 data on the assumption that "FB", F_1 and "LM" seed weights occur in F_2 in a 1:2:1 ratio, comes close to the observed mean, suggesting that a single allelomorphic pair of genes are primarily responsible for differences in seed weight. But although the peak of the F_2 curve is nearly coincident with the 1928 mean seed weight of "FB", there is no secondary mode agreeing with the mean for "LM", values are far more scattered at this end; the long straggling tail may represent a genuine transgression of the parental limit, or stunting of seed by undetected virus infection. Sowings to test this point have not been made. Or "scatter" here may have been introduced by the "LM" parents.

Whether or no the main segregation really is of the simplest type, there is no doubt that within both heavy and light-seeded classes the establishment of lines showing minor but definite differences is an accomplished fact (*cf.* pod breadth).

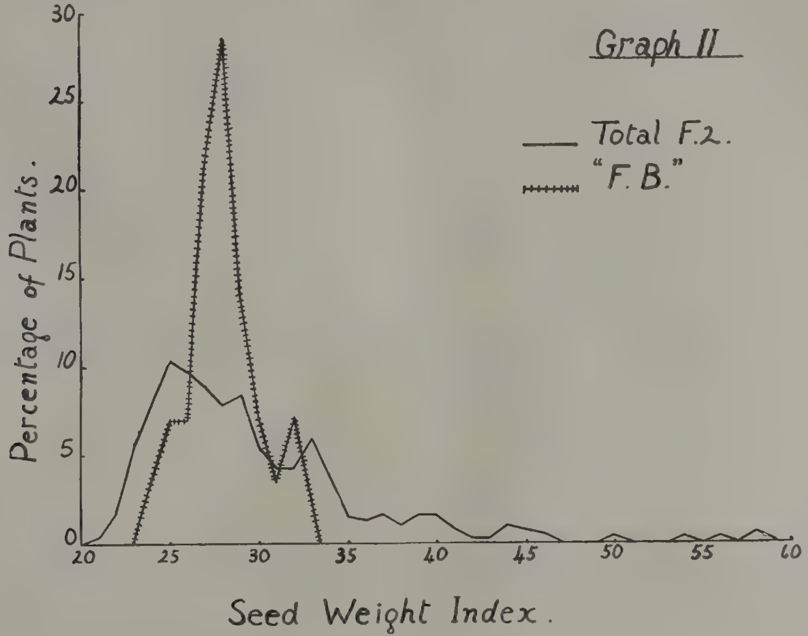


TABLE VI.

Seed weight. History of some lines.

F₂ (1929.)		1930.		1931.	1932.	
Family as whole.	Selected individual.†	Family as whole.	Selected individual.†	Family as whole.	Family as whole.	Designation of line.
	30	21·0	19	—	21	3.4.2.1
—	25	25·9	—	—	26	7.4.35
28*	—	25·0	—	27	26	"FB"
—	31	32·5	—	—	35	7.3.14
—	37	38·0	32·5	36	44*	4.1.31.4
—	29	29·5	25·5	32	30	7.4.8.5
35	—	32·5	—	38	36	"LM"

* Virus disease known to be present.

† Parent plant from which next season's crop derived.

The above table gives the seed weight history of various lines grown in 1932. It will be seen that the relative *order* of seed weight is generally unchanged by vicissitudes of season, although the actual weights may be considerably altered. 1930 was more favourable than 1931, for example.

Note that 3.4.2.1, which quite definitely exceeds "FB" in seed weight, owes its origin to a fairly small-seeded F_2 individual. 7.4.8.5, on the other hand, has arisen from a plant actually selected as being an unusually large-seeded "LM" type.

Such races as these are possibly the result of isolating various modifying or minor factors in a homozygous condition; or alternatively may be due to varying response of the major allelomorphs to different factorial interactions. It is hard to see, however, why 3.4.2.1 should produce super-large seed except as a result of carrying a definite factor or factors directly responsible; since it is an early broad podded form, a type understandingly ill-balanced from a physiological and autecological point of view, as compared with the parental combinations of characters.

Tschermak (22), in a cross between a *Pisum sativum* variety (large-seeded) and a *P. arvense* type (small-seeded), obtained an F_1 with seeds of intermediate size, and an F_2 which was classified into

arvense size	22
small medium	603 (or 613)
large medium	168 (or 158)
sativum size	3.

He suggests four pairs of allelomorphs as responsible. This wide cross is not very comparable to the case of "FB" × "LM".

The same author (23) also states that relatively large seed × relatively small gave an F_1 intermediate in size, and an F_2 series segregation by plant (*cf.* page 155), the larger parental type not being recovered. (It is possible that this was a seasonal effect.)

Lock (9) in his cross between "Telegraph" and "Native" pea found the F_1 to be intermediate between the parents in seed size.

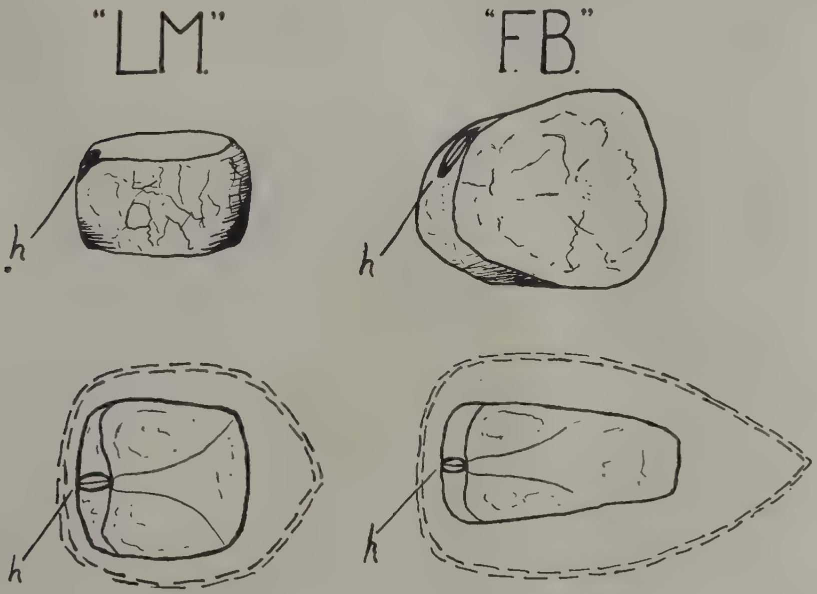
SEED SHAPE.

"Little Marvel", or at any rate the race thereof used as the parent of these hybrids, has seeds of the shape sometimes described as "cylinder section". This does give some idea of the characteristic form. The hilum is situated on the circumference of the "section", parallel to the long axis of the hypothetical cylinder.

The flat, or somewhat concave faces of the seed, corresponding to the "cut surfaces" of the "section", are therefore over the radicle in one instance, and the opposing edges of the cotyledons in the other. The distance through the two cotyledons is greater than their breadth.

This side to side compression is perhaps partially controlled by the degree of crowding in the pod, and is of course subject to modification, particularly in the case of the distal and proximal seeds.

"FB" seldom or never has seeds of this shape. Instead they are compressed in a direction laterally transverse to the length of the pod, so that the cotyledons are considerably broader than their combined thickness.



Semi-diagrammatic representation of the dry seeds.
Upper figures: In side view.
Lower figures: Transverse section of the pod with pea *in situ*. h. the hilum.

Seed from F_1 plants, and F_2 plants known to be heterozygous for pod breadth, perhaps vary in the direction of "LM" shape a very little more than "FB" peas, though in the main they resemble the latter, and variation is almost entirely inter-pod, all the peas in a pod being very similar in shape, size and colour.

As with seed weight, segregation occurs *by plant* in the F_2, so that a pea genetically of "LM" form may nevertheless be like "FB" superficially.

Lock notes the same type of phenomenon (10).

> "Some of the dimpled seeds on a heterozygous plant from dimpled × round gave rise to plants in which no trace of the dimpled (dominant) character is to be observed. . . . It is therefore a maternal character which becomes impressed upon the offspring in some way unconnected with heredity."

132 plants "FB" shape to 40 "LM" shape were scored in F_2, with about 10 doubtful cases, so that a single pair of allelomorphs seems to be responsible for the difference.

It is likely that "FB" shape is associated with especially high culinary quality.

Percentage of Two-flowered Racemes.

The proportion of potentially twin-podded peduncles is another of the characters influencing yield, and, like seed weight, is typical of practically important features in being difficult to work with. The very great variability which it shows is theoretically divisible into the portion with a definite direct genetical basis, and the remaining fluctuation. In practice it may be impossible to separate the two. Of first importance in causing fluctuation is the influence of diverse seasons; for example it will be seen from Table IX that the 1932 crop gave an almost uniformly higher yield of double-flowered racemes than the 1930 plants. Further analysis of the influence of season awaits experimental attack. A first hypothesis would be that any combination of environmental circumstances favouring vigorous growth of the plant as a whole would also increase the percentage of double-flowered racemes, but this is not always true. The 1930 crop was far more vigorous and healthy than that of 1932. It is clear that favourable conditions can only be effective during the period at which the rudimentary racemes are being laid down at the growing point of the stem, comparatively early in the plant's life. Liberal treatment at, or just before this period, might be expected to act in a manner reminiscent of the "flushing" of breeding ewes. Casual observation tends to confirm this.

It is therefore natural that time of sowing in any particular year will exert considerable influence on the feature under discussion. Mr. Boyes informs me of an extreme instance; where a normally very paired-flowered variety produces single flowers almost exclusively, when autumn sown. The "critical period" will of course occur at different dates in the case of varieties of different habit, and rapidity of development. The fact that a season favourable to one variety may act adversely on another, sown at the same time, is therefore understandable. The low "doubles" percentage of "FB" in 1932, a season generally favourable for "doubles" production, is a case in point

(Table IX). The comparison of crops grown side by side is therefore unusually unreliable ; and the homologizing of crops in different years by means of a " seasonal factor ", obtained from the two sets of figures for a standard variety, unsafe.

In addition to the usual medley of edaphically, meteorologically and other environmentally determined variation, the plant to plant fluctuation under the most uniform conditions obtainable seems unusually great. There is some suggestion that twin or single-flowered racemes tend to occur in " runs " along any axis, as though a double " pattern " once established in the stem apex tended to persist. The same sort of thing is sometimes observable in connection with phyllotaxy. If this really is the case the influence of initial " chance " in producing divergence from the general average will be magnified.

TABLE VII.

1928 Crop (data by courtesy of Mr. Boyes).

Pe_2.	No. of Plants scored.	No. of Double Racemes.	No. of Single Racemes.	Per cent. of Double Racemes.
" FB "	468	389	1,541	20·1
" LM "	1,213	3,774	2,344	61·7
F_1 (except X.1) ..	18	3 " chiefly single "	2 " double and single " 13 " chiefly double "	
F_1 of X.1 ..	4	1 " all single "	2 " chiefly single " 1 " double and single "	

TABLE VIII.

1929 Crop.

Pe_2.	No. of Plants scored.	No. of Double Racemes.	No. of Single Racemes.	Per cent. of Double Racemes.
" FB "	28	11	117	8·6
F_2 (except of X.1)	319	1,015	827	55·1
F_2 of X.1 ..	42	48	155	23·7

" LM "—No accurate data : estimated about 65 per cent. Double Racemes.

The above figures for the 1929 crop are derived from an examination, not of the whole plants, but of the main stems only. The percentage of " doubles " on the branches is usually rather less than that for the primary axis, hence the percentages should be rather lower to be comparable with other years. (This draws attention to the complication that conditions favourable for branching tends to lower the percentage of double racemes ; it would probably be best always to consider main stems only, as being most likely to show the full potentialities of the plant.)

Care has to be taken in scoring the dried plant not to miss the scar denoting an aborted second flower on a single *podded* peduncle.

Only 1 plant in 28 of "FB" had more than 33 per cent. of double racemes, and in view of this fact, and the 1929 figure, the F_2 plants were grouped into those with above and including, and below $33\frac{1}{3}$ per cent. "doubles".

The very high standard deviation of the character, accentuated by some plants bearing only three or four scorable racemes, makes it unlikely that genetically "high" and "low" classes will not transgress this line in both directions.

The groups so defined should nevertheless give a fair approximation to the "LM"-like and "FB"-like types. Both F_1 and F_2 results indicate that the former is more or less dominant.

The F_2 (excluding X.1 derivatives), is therefore divided into 232 "high per cent. double" and 94 "low per cent.",

$$244{\cdot}5 : 81{\cdot}5 \text{ are in the ratio of } 3:1.$$

The observed difference from this proportion is 12·5, and the standard error 7·82. It is therefore possible that a single pair of allelomorphs are responsible for the difference in the percentage of paired flowers borne by "FB" and "LM". These genes might be symbolized "D" and "d".

X.1 gave an F_2 segregation of

14 "high per cent. double" : 28 "low per cent. double".

Plant breeding considerations determined that most of the F_3 families were raised from plants with the highest number of double racemes. In the absence of a sufficient number of families raised from lower-percentage-double F_2 plants little further light was thrown on the genetic behaviour of double poddedness.

Table IX shows that 1930 was a very unfavourable season for "doubleness" (see also page 160). The general agreement in the order of the different stocks in 1930 and 1932 is good; that of the four best, and the three worst remains unchanged. It is evident that within the relatively high-percentage "doubles" group the fixing of strains differing in a minor but definite manner is possible.

The single F_3 plant 4.1.29.4 was evidently a case of fluctuating, rather than directly genetically determined low "doubles" percentage, or the result of especial susceptibility to an adverse season; that is, selection was here ineffective. On the other hand, selection in 7.3.10 resulted in the production of divergent lines derived from plants 5 and 6 of the family. This is quite typical of the manner in which the very great fluctuation of the character is effective in over-riding or obscuring the directly genetically determined potentiality of the plant.

TABLE IX.

Proportion of two-flowered racemes. History of some lines.

1929.		1930.					1931.				1932.			
F₂ plant.		F₃ Family as a Whole.		Relative Order of "Doubleness" in 1930.	Single F₃ Selected Plant.†		Family as a Whole.		Single Selected Plant.†		Family as a Whole.		Relative Order "Doubleness" in 1932.	Designation of Line.
No. of "Doubles".	No. of "Singles".	D.	S.		D.	S.	D.	S.	D.	S.	D.	S.		
7	0	273	273	1	—	—	—	—	—	—	160	70	1	4.1.32
7 10	0 3	63	115	2	18	18	High % double	—	—	—	268	162	2	4.1.31.4
6	1	80	160	3	—	—	—	—	—	—	250	250	3	7.2.37
9	0*	150	346	4	—	—	—	—	—	—	100	120	4	7.3.14
5	0	100	300	5	4	3	189	153	—	—	100	200	6	7.4.8.5
7	1	106	420	6	16	26	High % doubles	—	21	22	159	296	—	7.3.10.5.1
,,	,,	,,	,,	,,	,,	,,	,,	—	24	7	202	199	—	7.3.10.5.2
,,	,,	,,	,,	,,	,,	,,	,,	—	17	20	212	333	—	7.3.10.5.3
,,	,,	,,	,,	,,	0	37	Fair % doubles	—	10	14	124	712	—	7.3.10.6.2
,,	,,	,,	,,	,,	,,	,,	,,	—	?	?	83	388	—	7.3.10.6.5
,,	,,	,,	,,	,,							880	1,928	8	7.3.10 (Total)
5	3	72	562	8	8	17	—	—	—	—	140	300	7	6.5.15.14
		(Above are all narrow-podded forms)												
7	0	79	553	7	0	25	Fair % doubles	—	15	13	102	200	5	4.1.29.4.3
6	0	5	184	9	—	—	—	—	—	—	25	100	9	7.4.35
3	0	2	91	10	0	15	—	—	—	—	6	180	—	3.4.2.1
,,	,,			—	0	20	—	—	—	—	4	350	—	3.4.2.10
,,	,,										10	530	10	3.4.2 (Total)
					not including the 3d plant									
0 and all single on branches	5	3	235	11							0	320	—	4.1.3
	,,	,,	,,	—	0	20	—	—	0	19	2	150	—	4.1.3.8.4
	,,	,,	,,	—	,,	,,	—	—	0	8	0	130	—	4.1.3.8.6
	,,	,,	,,								2	600	11	4.1.3 (Total)
Total 11	117	—	—	—	—	—	—	—	—	—	3	410	—	"FB"
		(Above are all broad-podded forms)												

* All "doubles" on branches, also.

† Selected as parent of next generation.

As expected, pursuit of a *consistently** double-flowered race seems vain. It is noteworthy, on the other hand, that of four plants sown as consistently single-flowered (branches as well as main stem examined), three gave families without a single double raceme; the fourth family contained a single plant with three double as well as single racemes.

Tedin (15) points out the great modifiability of "doubleness" and "singleness". He found that plants:

exclusively "single" gave 64·5 per cent. single-podded plants,
"single", also "doubles" ,, 55·9 ,, ,, ,, ,, ,,,
with "doubles" proponderating ,, 32·0 ,, ,, ,, ,, ,,.

TIME OF FLOWERING AND FRUITING.

The influence of environmental conditions on the rapidity of development of a plant requires no stressing.

If "LM" and "FB" are sown together, the former develops its first pods and contained seeds to an extent called "fitness to pick" (for culinary purposes), about nine or ten days before the latter.

The average difference in the date on which the first flower opens is, however, only about six days, so that the post floral development of "LM" takes, therefore, four days less.

The F_2 plants were each labelled (by Mr. Boyes, to whom my thanks are due for the data so provided) with the dates on which the first flower opened, and on which the resulting pod reached the arbitrary degree of development; scoring taking place at three day intervals.

These two dates may conveniently be indicated by "FF" and "FP".

With the exception of X.1 (see page 173), various families behaved in similar fashion, and the sum of the data for FF is as follows:

TABLE X.

FF date ..	12/6	15/6	18/6	21/6	24/6	27/6	30/6	Total
No. of Plants:								
Total F_2 except X.1	11	29	126	85	63	11	1	326
F_2 from X.1 ..	—	—	6	20	8	—	—	34
"FB" ..	—	—	10	9	7	1	—	27

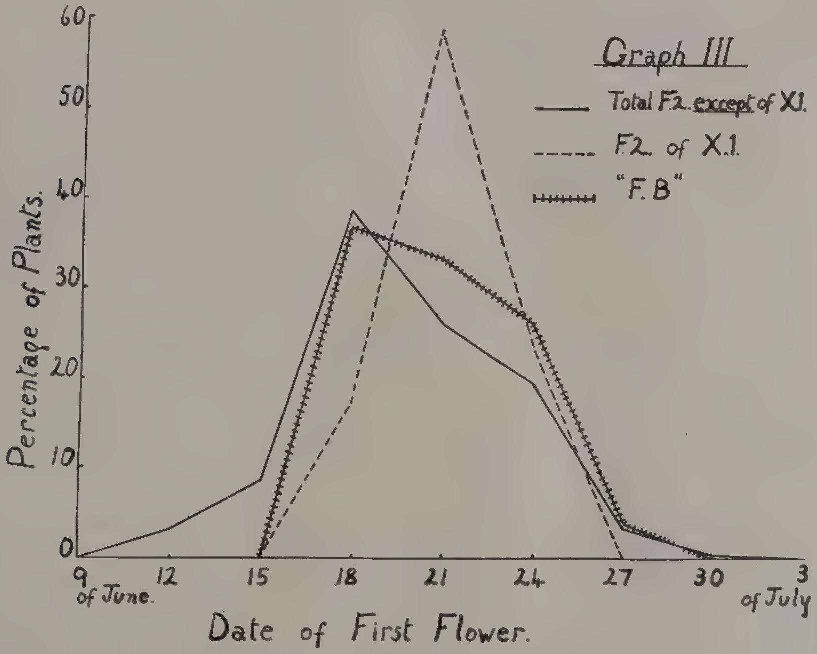
(See also Graph 3.)

Individual plant records for "LM" unfortunately were not obtained, but that variety commenced to flower about June 12th; the bulk had opened their first flowers by the 15th, and the last ones were about three days later. The parental types therefore overlap in this respect, and the range of fluctuation of either is at least equal to the average difference between them. Any hybrid intermediate genotype or genotypes will presumably have a similar spread so that all F_2 classes will overlap to a very great extent.

---

* So far only a form seen under the name "Pisum Jordani" (probably *not P. Jomardi* (Schrank.)) approaches consistent doubleness; two doubtfully single racemes occurred in about one hundred examined.

Any classification of F_2 plants into early, intermediate and late, according to the actual date of FF, will be misleading.



The mean date for the whole F_2 is 19·8/6, *later* than if a single factor for "FB" time of flowering, averaging six days later than "LM", was *completely* dominate to earliness. Mr. Boyes noted that the dominance of comparative lateness was not complete in F_1. It is therefore fairly certain that several factors influence FF date. The transgression of the parental limit in the direction of increased lateness, apart from being the normal attribute of a much larger sample, may be the result of somewhat diseased plants, with an abnormally delayed FF, being included in the records, owing to their condition being unrecognized in the dried state; and a few plants with damaged main stems, and therefore a delayed FF on a branch, may have escaped being discarded. The "disease effect" was much more marked in 1930, but so far

TABLE XI.

Distribution of FF date in families derived from F_2 parents of given FF date.

Family.	F_2 (1929) FF Date	F_3 (1930) Dates.												
		16/6	18	20	22	24	26	28	30	2/7	4	6	8	
3.4.2 ..	12/6	—	2	6	9	5	—	—	—	—	—	—	—	
4.1.2 ..	,,	—	2	4	13	3	1	—	—	—	2	—	—	
4.1.3 ..	,,	—	1	7	4	7	4	—	—	—	—	—	—	
5.1.2 ..	,,	—	—	19	3	—	—	—	1	—	1	—	—	
5.1.3 ..	,,	—	—	—	—	17	2	—	3	—	—	—	—	
7.3.1 ..	,,	—	—	6	3	6	5	—	—	—	2	—	—	
Total for ..	12/6 families	—	5	42	32	38	12	(3)*	4(1)*	(3)*	5(2)*	—	—	128
3.1.3 ..	15/6	—	—	1	4	1	23	—	5	—	8	—	2	
3.1.4 ..	,,	3	2	—	3	12	5	—	—	—	—	—	—	
3.4.5 ..	,,	—	—	6	6	25	6	—	2	—	—	—	—	
6.5.5 ..	,,	—	3	12	13	1	—	—	—	—	—	—	—	
7.2.3 ..	,,	—	4	16	2	1	—	—	—	—	—	—	—	
Total for ..	15/6	3	9	35	28	40	34	(4)	7(3)	(6)	8(2)	—	2	166
2.1.9 ..	18/6	—	—	—	—	1	10	—	8	—	2	—	—	
3.1.14 ..	,,	—	—	—	—	5	6	—	2	—	1	—	—	
3.4.15 ..	,,	—	—	4	1	3	3	—	—	—	—	—	—	
4.1.7 ..	,,	—	2	1	2	9	2	—	2	—	—	—	—	
5.1.18 ..	,,	—	—	—	—	5	14	—	13	—	5	—	—	
6.5.10 ..	,,	—	—	—	7	15	8	—	12	—	7	2	—	
6.5.12 ..	,,	—	—	—	1	6	17	—	1	—	—	—	—	
6.5.15 ..	,,	—	—	—	—	—	12	—	5	—	6	2	—	
6.5.19 ..	,,	—	—	—	—	2	8	—	5	—	4	—	2	
7.2.20 ..	,,	—	—	3	2	2	12	—	3	—	2	—	—	
7.3.9 ..	,,	—	—	1	2	2	11	—	5	—	1	—	1	
7.3.10 ..	,,	—	—	—	—	—	15	—	9	—	1	—	—	
7.3.14 ..	,,	—	1	6	2	21	14	—	5	—	—	—	—	
7.3.15 ..	,,	—	—	—	2	3	10	—	6	—	2	—	—	
7.4.8 ..	,,	—	—	—	—	18	20	—	5	—	1	—	1	
7.4.15 ..	,,	—	—	—	—	—	16	—	3	—	1	—	—	
Total for ..	18/6	—	3	15	19	92	178	(56)	(28) 84	(22)	(11) 33	4	4	432
3.4.19 ..	21/6	—	—	—	—	—	9	—	2	—	2	—	—	
3.4.21 ..	,,	—	—	—	—	1	19	—	4	—	—	—	—	
3.4.23 ..	,,	—	—	1	5	3	11	—	1	—	—	—	—	
4.1.27 ..	,,	—	—	3	—	3	7	—	1	—	1	—	—	
4.1.29 ..	,,	—	—	3	3	3	9	—	15	—	5	3	1	
6.5.32 ..	,,	—	1	3	5	13	10	—	8	—	2	1	1	
6.5.33 ..	,,	—	—	—	5	6	9	—	4	—	1	—	—	
7.3.23 ..	,,	1	—	5	5	7	12	—	17	—	2	—	—	
7.3.27 ..	,,	—	—	—	—	1	3	—	7	—	5	1	—	
7.4.18 ..	,,	—	—	—	—	—	11	—	8	—	2	—	1	
7.4.23 ..	,,	—	—	—	—	1	7	—	35	—	5	1	—	
Total for ..	21/6	1	1	16	23	38	107	(68)	(34) 102	(17)	(8) 25	6	3	322
4.1.31 ..	24/6	—	—	—	—	1	11	—	4	—	4	—	—	
4.1.32 ..	,,	—	—	—	—	5	11	—	4	—	2	—	—	
5.1.36 ..	,,	—	—	—	1	2	3	—	5	—	4	1	—	
7.4.27 ..	,,	—	—	1	2	2	12	—	3	—	3	1	—	
7.4.34 ..	,,	—	—	1	4	3	9	—	4	—	3	—	—	
7.4.35 ..	,,	—	—	—	—	—	—	—	6	—	16	—	—	
Total for ..	24/6	—	—	2	7	13	46	(17)	(9) 26	(21)	(11) 32	2	—	128

(See Graphs IV and V.)

* See text for explanation of figures in brackets.

no case of genetically determined transgression of the parental limits (either early or late) has been found amongst the hybrid segregates tested.

Plants selected on economic grounds were sown to provide the partial F_3. Table XI shows the distribution of FF date in the resulting families, together with the FF date of the parent plant. It will be seen that scoring was not carried out on June 28th and July 2nd, so that plants which should have been labelled with these dates were included in the 30/6 and 4/7 classes respectively. About two-thirds of each of these latter classes therefore belong to the preceding column. No attempt has been made to smooth the distribution in this manner in the table. (Except for the totals, since failure to do so there would leave the derived graphs (4 and 5) enormously distorted.)

The number of plants in the F_3 family was frequently insufficient to give a dependable idea of the real distribution of FF date; originally either 50 or 25, it was further reduced in many cases by the omission of damaged or diseased plants, the latter being so numerous in some families as to leave only a few plants, not worth inclusion in the table.

TABLE XII.

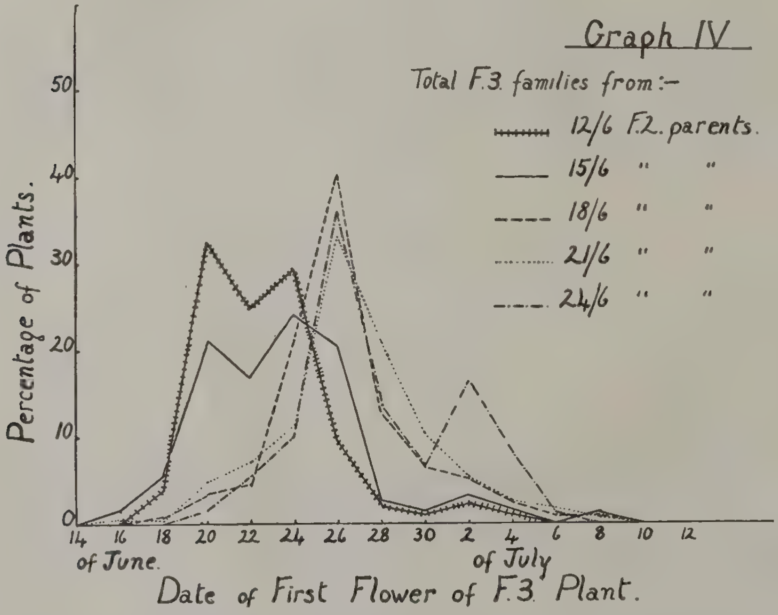
Mean F_3 FF dates for different parental F_2 FF dates.

FF Date of F_2 Parent Plants.	Total No. of F_3 Plants.	Mean FF date of F_3.	Standard error of Mean.
12/6	128	22·624/6 ± 3·428	0·304
15/6	166	23·542/6 ± 3·860	0·300
18/6	432	26·366/6 ± 3·246	0·156
21/6	322	26·782/6 ± 3·626	0·202
24/6	128	27·860/6 ± 4·004	0·354

Table XII gives some idea of the "regression" of the F_3 groups towards the general mean; an F_2 range of 12 days between the means has become reduced to one of slightly more than 5 days. In view of the comparatively wide spread of a line homozygous for FF date, the F_2 plants flowering on any one date must have included several different genotypes, so that this "regression" is to be expected. Nevertheless it will be seen that the means of the F_3 descendants retain the correct order, although statistically speaking the difference between 12/6 and 15/6, 18/6 and 21/6, and 21/6 and 24/6 groups are not very significant. The curves (Graph 4) emphasize the similarity of the first two groups and of the last three.

Graph 5 shows 12/6 and 15/6 classes added together, and the remainder likewise grouped. Since lateness is partially dominant, the 18/6 and 21/6 classes would have been expected to include the majority of the more heterozygous plants.

It is therefore surprising that the early groups, rather than they, should show such an obvious mark of segregation as a twin mode. The number of plants in the first two groups is comparatively small, however, and on examination of the data set out in Table XI it is seen that the second peak of the curve is mainly due to the influence of three families, 5.1.3, 3.1.3 and 3.4.5. These "intermediates", presumably included in the early classes owing to parental fluctuation, are separated from the remaining families in Graph 5, when the 12/6 and 15/6 curve is resolved into two of more normal appearance. Such families in the 18/6 group as 3.4.15, 6.5.12 and 7.3.14 apparently belong to the similar genotype as the "intermediates" mentioned above, but small families, probably a number of genotypes, and a fairly large total number of families combine to give a superficially "normal" type of distribution, which is nevertheless in reality compound.



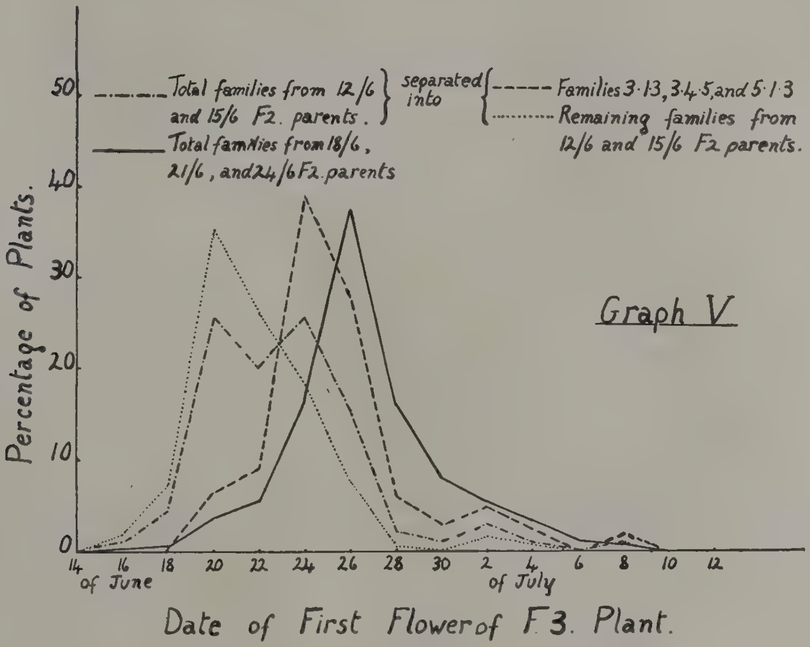
Finally, it may be remarked that the range of FF is noticeably smaller in some families than others, indicating a comparatively homozygous condition.

The high variability of the character in proportion to the difference between the parental lines, the length of the 3-day scoring interval employed in the examination of F_2, and the difficulty of preventing distortion of these results by failure to eliminate all effects of disease, would make any attempt at factorial analysis mere guesswork. The establishment in F_4 and F_5 of true breeding lines of various intermediate mean FF dates proves that no simple scheme can be adequate ; if such proof is necessary.

Mendel (11) found in a cross between early and late flowering varieties that the F_1 was intermediate in character ; the F_2 gave an unclassified range.

Lock (9), in his cross of "Native" pea and "Telephone", obtained an F_2 which he arbitrarily divided at 5-day intervals into

63 Early : 186 I : 279 Late.



Denaiffe (2), Terasvuori (19), Tedin (15) and Keeble and Pellew (7) have all pointed out that the flowering time is correlated with the number of sterile nodes preceding the fertile one. Node number has not been studied in this investigation, but in F_2 height to the first fertile node, which is partly a function of node number, was definitely correlated with FF date (page 182).

Tedin (16) crossed two varieties which flowered at about the same time, and obtained transgressive segregation in both directions.

Tschermak (20) gives an F_2 segregation of 22 or 23 E : 191 I : 18 L, but without defining the limits of these classes.

Elsewhere (21) he describes an F_1 as intermediate, tending to late; the F_2, divided into groups flowering with the parents, and an intermediate class, consisted of 60 E : 190 I : 88 L, which is compared with the 63·3 : 190 : 84·4 of a theoretical 3.9.4[*] ratio. Tschermak assumes that a factor, A, causes intermediate, tending to late, flowering, and another, B, accelerates flowering, acting only when A is present; the early parent then being AABB.

Wellensiek (25) very mildly points out that as Tschermak's figures were obtained by adding crosses of different varieties, that individually did not show the expected segregation, too much reliance cannot be placed upon them!

The F_3 results obtained only agreed moderately well with expectation.

Keeble and Pellew (7) had the advantage of working with two varieties differing in flowering date by at least a month. Their scoring intervals, or else the behaviour of their plants, seems to have been very irregular. Witness, for example, the flowering of twenty-three plants of "Bountiful" on June 2nd, then no more until five are recorded for June 10th.

It is stated that lateness is dominant to earliness, but that flowering is accelerated by the factor for long internodes and also by that for thinness of internode.

Nevertheless, if, as they postulate, only a single dominant factor causes lateness, $\frac{3}{16} \times \frac{3}{4} \times 192$ F_2 plants (i.e. 27), should have been as late as "Autocrat", whereas only one flowered on the date that "Autocrat" was only just commencing to bloom. (In the text, June 30th and not July 6th is given.)

It seems distinctly probable that Keeble and Pellew's interpretation erred on the side of simplicity; and that more factors were really concerned.

Hoshino (6) found lateness imperfectly dominant in an F_1; a distinct minimum frequency class occurred in F_2; and was arbitrarily used to divide the "earlier" from "later", being itself placed with the earlies; the ratio so obtained being 258 : 557. He suggests that this represents a 6 : 10 segregation, according to the following scheme:

A causes late flowering.
a early, epistatic to b.
B accelerates FF, b retards earlies by some days.

---

[*] The F_2 expectation is then given as:—

AABB	early	(1)	Aabb	intermediate to late	(2)
AABb	early to intermediate	(2)	aaBB	late	(1)
AAbb	intermediate to early	(1)	aaBb	late	(2)
AaBB	intermediate	(2)	aabb	late	(1)
AaBb	intermediate to late	(4)			

If the F_1 heterozygote AaBb is intermediate to late, A must be dominant and B recessive. AABb should therefore be intermediate to late (unless B becomes dominant in the presence of homozygous A); AAbb is intermediate to late by definition, and AaBB early. A 3 : 9 : 4 ratio is therefore arrived at, but not as Tschermak states.

F_2 then

aabb	(1)	early	AAbb	(1)	late
aaBB	(1)		AABB	(1)	
aaBb	(2)		AaBb	(4)	
Aabb	(2)		AaBB	(2)	
			AABb	(2)	

Wellensiek (25) points out, however, that the Aabb class should be late, not early.

Again F_3 results gave only moderate agreement with theoretical expectations.

Wellensiek (26) crossed the late variety, " Bliss Abundance ", with the early " Haarsteegsche ", obtaining an F_1 intermediate in FF date.

The F_2 plants were classed as early ; intermediate, if like the F_1 ; or late, in the proportions 120 : 292 : 99 ; *cf.* a theoretical 128 : 288 : 96.

Table XIII.

Wellensiek's results condensed.

Date.	Range.				
June 22	♂ parent	120 " Early "	iill (1) iiLl (2) iiLL (1)	4	F2
" 23					
" 24					
" 25					
" 26					
" 27					
" 28	F1	292 " Inter."	Iill (1) IiLL (2) IiLl (4) Iill (2)	9	
" 29					
" 30					
July 1					
" 2					
" 3	♀ parent	99 " Late "	IILL (1). IILl (2)	3	
" 4					
" 5					
" 6					
" 7					
" 8					

He reversed Tschermak's factorial scheme :

If = Inter., if = early.
Lf = retards If ; in itself ineffective, ∴ 4 : 9 : 3 ratio expected,
cf. Tschermak's 3 : 9 : 4.

The definition of " early " and " intermediate " is, however, more or less arbitrary. A fairer method would be to class those FFs falling within the parental ranges as early or late, and all between intermediate.

The ratio E : I : L would then be 27 : 385 : 99.

The assumption of two factors A and B, one fully dominant, the other half dominant, their effect being cumulative:

Early parent = aabb
Late parent = AABB
F_1 = AaBb

would then explain the late-intermediate position of the F_1, and in F_2 would require an ideal ratio of

32 = as early parent
384 = intermediate dates
96 = as late parent ;

an even closer agreement with the observed results than is given by Wellensiek's scheme!

It may be said that none of the various factorial schemes advanced are at all convincing.

It has been shown that a 6-day interval depends on several factors, or factorial inter-relations ; how complicated the question of FF date in garden peas in general must be is realized in view of the existence of numerous lines and varieties differing with regard to the character, and together comprising a very wide range of genetically determined variation.

Sufficiently accurate data for the FP date of the F_2 plants was not available, so that the nature of the segregation for FP-FF interval could not be determined.

Foliage Colour.

The slight but definite distinction between the parental shades of green is best observed in the comparatively young plants when they are only a few inches high. Later not only do the individual plants (spaced six inches apart) grow together, but the incidence of "mosaic" disease may obscure the normal colour. A badly virus infected "dark" plant frequently produces pale foliage thenceforward, apart from the paleness due to the actual mosaic netting.

F_2 families raised from X.1, and also from plant No. 2 of X.4, were abnormal in being "FB" colour throughout (see page 175). All other families showed segregation into relatively darker and paler plants, corresponding to the colours of the two parental types.

533 plants proved to have been labelled "D"
and 153 ,, ,, ,, ,, ,, ,, "P"

in the field by Mr. Boyes.

An exact 3:1 ratio is 514·5:171·5.
The observed difference is 18·5,
the standard error = 11·34,

so that the probability of the observed results being the expression of a normal monohybrid segregation is of the order of one in ten.

Some indication that pale plants tend to fall victims to disease more readily than dark ones is a probable explanation of this moderately large deficit. The presence of a single allelomorphic pair of genes for foliage colour (which may be symbolized "Dl" and "dl") was confirmed by the partial F_3.

Of 17 "pales" sown, 16 bred true for light foliage, and 1, which must have been a scoring mistake, gave all dark progeny.

Of the 39 "darks" sown, 14 bred true for that colour, and 25 segregated in ratios of approximately 3:1. Early and severe virus infection supervened in time to prevent accurate counting of the segregants.

The F_1 plants (except the X.1 family) were noted by Mr. Boyes as of "LM" colour, but X.7 appeared to be slightly darker than the rest. It is therefore of interest that one plant in the F_2 from X.7, Plant 2, and two plants from X.7, Plant 4, were scored as being "VD" (very dark), i.e. darker than "LM". Two of these extra dark plants seem to have bred true for this feature up to F_4; the third threw a percentage of "pales" in F_3; but it was hardly possible to score individual plants as "VD" or "D" with certainty, although in mass the difference is obvious enough.

Hence no attempt has been made to determine the relationship of "VD" and "D". The former colour is in all probability derived from the "LM" parent of X.7 (see Preface).

Under optimum lighting conditions the plants of some pale cultures were seen to be of two alternative shades.

The only previous work on foliage colour (apart from the question of wax covering and glaucousness) has been concerned with a much more striking contrast between a definite yellowishness, and "normal" green (27). This divergence also was found to depend on a single allelomorphic pair of factors.

THE ABERRANT BEHAVIOUR OF X.1.

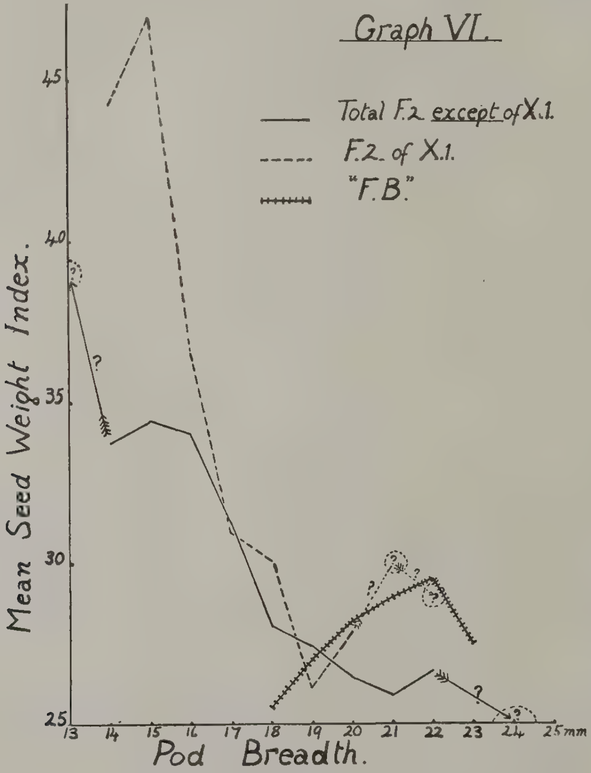
The distinctive behaviour of X.1 was mentioned in the Introduction.

Table I, and Graph I for pod breadth, or Table V for seed weight, fails to show anything distinctive about its segregation; while its behaviour with regard to seed shape is similar to that of the other hybrids.

The proportion of double racemes it bears is, however, comparatively very small (Tables VII, VIII); the F_2 of 14 plants with high per cent. "doubles": 28 low per cent. "doubles", is very significantly at variance with 232 high per cent.:94 low per cent. for the rest of the hybrids. In this respect X.1 is, if anything, closer to "FB" than to the general F_2. A few small F_3 families have no more "doubles" than "FB".

The curve for X.1 in Graph 7 shows that although pod breadth segregation is "normal", and "double" percentage at least higher than "FB", yet the relation between the two characters is (probably uniform, showing no correlation at all, with sufficient numbers) of the type shown by the latter!

Table X shows that no segregation for FF data occurred in F_2 (and has never done so in succeeding generations), the family again behaved like "FB".

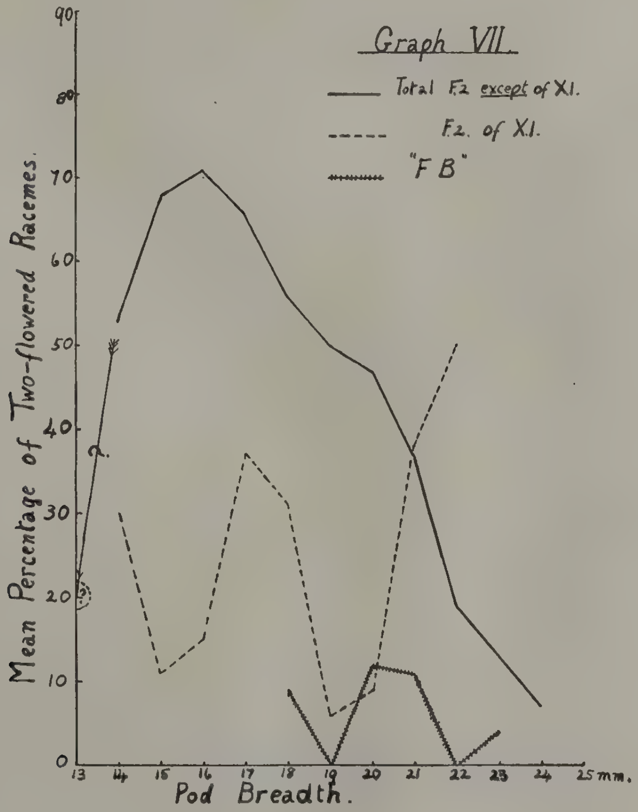


Reference to Graph 8, however, shows that X.1 behaves like the other hybrids, rather than "FB", in that the later pods are the broadest. This suggests that although the range of FF date corresponds with that of the female parent, some sort of suppressed or "telescoped" segregation is nevertheless taking place.

The small numbers render this uncertain, however. The time required for pod development also seemed to be similar to that for "FB".

Finally, F_1, F_2 and succeeding generations have always been of "FB" green.

X.1 plants and their descendants have therefore behaved as "normal" hybrids with regard to pod breadth, seed size and shape; more nearly like "FB" in "doubles" percentage, and identical with "FB" in flowering and fruiting period and foliage colour.

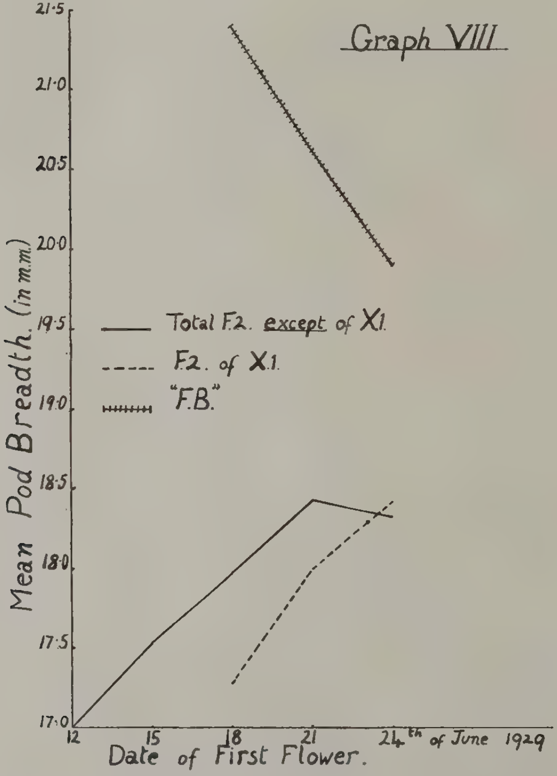


The simplest way to account for these facts would be to assume that the "LM" parent of X.1 was not typical of that variety (see Introduction) but contained the factors for foliage colour, "singleness", and period proper to "FB". In view of the difference in earliness of flower and fruit between "LM" proper and "FB" being in all probability dependent on several factors, the complete identity of X.1 with "FB" in this respect is rather remarkable.

On the other hand it is not easy to visualize any cytological abnormality affecting the *several* hybrid zygotes of X.1 in a manner leading to these results.

THE CORRELATION OF CHARACTERS.

Few of the various features of these peas segregate in a completely independent manner.



There is some doubt as to the relationship existing between pod breadth and seed weight. In general, broad pod and heavy seed are closely associated (Table XIV and Graph 6). Lock (9) suggested the possibility of a mutual influence of seed size and pod breadth.

The correlation is, however, far from absolute; whether this is because different but linked factors are responsible for the major pod and seed segregations, or whether the divergences are due to modifying factors alone, it is impossible to say. A few trial sowings from the large seeded, narrow-podded quadrant have yielded at least one line retaining this combination of features to a moderate extent; others seem to indicate that the parent F_2 plant owed its position to an excessive degree of fluctuation.

In one F_3 family a curious phenomenon was noted which may have some bearing on this point.

TABLE XIV.

Correlation between pod breadth and seed weight of the F_2 (*except of X.*1).

Pod Breadth.

Seed Weight Index.	13	14	15	16	17	18	19	20	21	22	23	24	Totals.
20	—	—	—	—	—	—	—	—	—	—	—	—	
21	—	—	—	—	—	—	—	1	—	—	—	—	1
22	—	—	—	—	—	1	1	3	—	1	—	—	6
23	—	—	—	1	1	1	2	6	3	—	—	—	14
24	—	—	—	—	2	6	7	4	2	1	—	—	22
25	—	—	—	1	3	5	5	13	2	1	—	2	32
26	—	—	—	—	2	6	7	12	2	1	—	—	30
27	—	—	1	2	2	7	4	10	3	1	—	—	30
28	—	—	3	3	5	6	1	7	1	—	—	—	26
29	—	—	2	4	1	9	5	3	1	—	—	—	25
30	—	1	—	5	1	5	5	1	—	—	—	—	18
31	—	—	—	5	2	2	1	3	1	1	—	—	15
32	—	1	3	3	—	4	3	—	—	1	—	—	15
33	—	—	3	4	4	2	2	3	—	—	—	—	18
34	—	1	2	1	5	2	1	1	—	—	—	—	13
35	—	—	2	2	1	—	1	1	—	—	—	—	7
36	—	—	2	—	2	—	—	—	—	—	—	—	4
37	—	—	—	2	3	1	—	—	—	—	—	—	6
38	—	—	—	2	1	—	—	—	—	—	—	—	3
39	1	1	—	2	1	—	—	—	—	—	—	—	5
40	—	—	3	2	2	—	—	—	—	—	—	—	7
41	—	—	—	3	—	—	—	—	—	—	—	—	3
42	—	—	—	1	—	—	—	—	—	—	—	—	1
43	—	—	—	1	—	—	—	—	—	—	—	—	1
44	—	—	2	1	—	—	—	—	—	—	—	—	3
45	—	—	1	1	—	—	—	—	—	—	—	—	2
46	—	—	—	2	—	—	—	—	—	—	—	—	2
Totals	1	4	24	48	38	57	45	68	1	7	—	2	309

Mean Seed Weight $= 29 \cdot 414 \pm 5 \cdot 33$
Mean Pod Breadth $= 18 \cdot 126 \pm 1 \cdot 95$
Correlation Coefficient $= 0 \cdot 49$ approx.

All the plants were narrow-podded to eye, but frequently rather large for this class, and with approaching maturity had a remarkable swollen, cylindrical look, apparently due to very tight packing of the contained peas. On *every* plant one or more pods, about fourteen days after the opening of the flower, became extraordinarily well filled, and burst open rather raggedly, usually down the ventral suture, leaving the large immature seeds protruding somewhat. Within a day or two these expanded more, the testas split, and with a rapidity depending on weather conditions, shrivelling and attack by moulds followed. As the better developed, earlier pods were usually the ones to burst in this manner, the seed weight index of 31 is probably not really typical of the family. No obvious reason for the seed becoming literally too large for the pod could be found. A small F_4 raised the next year showed the peculiarity to a much smaller extent ; and seed weight numbers were not obtained.

Otherwise it would be tempting to suppose that this abnormality represents the crossover combination, fully large seed and narrow pod!

The impossibility of fixing limiting values for the F_2 seed weight genotypes precludes the calculation of even approximate linkage values for the major seed weight and pod breadth factors.

Whatever the relation of the main factor or factors for pod breadth and seed weight may be, it is certain that at least some of the minor variations can be independently inherited. The super-large seeded race X.3.4.2, for example, has pods barely as broad as "FB". The considerable increase in the standard errors of the various pod breadth classes in Table XV, as compared to those for "FB" (BB), F_1 (Bb) and "LM" (bb) in 1928 (Tables III and IV) may be taken as an indication of such segregation of factors modifying seed weight, independently of pod breadth.

TABLE XV.

Seed weight index of F_2 plants of proven pod breadth constitution.

Constitution for Pod Breadth.	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
BB ..	—	1	1	2	3	2	2	2	2	2	1	—	—	—	—	—	—	—
Bb ..	—	—	—	—	4	5	4	1	2	3	—	1	1	—	—	—	—	—
bb	—	—	—	—	—	—	—	—	3	2	4	2	—	1	3	—	—	1

Constitution for Pod Breadth.	Mean.	S.E. of Mean.
BB	25·61±2·52	0·59
Bb	26·57±2·30	0·50
bb	31·00±2·57	0·64

Since high seed weight was given selection value in choosing the above parent plants of F_3, the figures will not be fairly representative of the pod breadth classes as a whole. This is especially true of the bb group, the mean seed weight of 31·0 being much higher than that for narrow-podded plants as a whole. In general, however, the table shows the close alliance between pod breadth and seed weight.

Note also that the difference in mean seed weight between BB and Bb classes, although statistically insignificant, hints at the incompleteness of dominance of heaviness (already demonstrated by comparing F_1 and "FB") running parallel to the incomplete dominance of broad pod.

"FB" shape is almost invariably combined with broad pod (and so with large seed), so that it was at first thought to be determined by the same gene. Examination of one F_3 family, however, disclosed two narrow-podded plants, the small seed of which was very definitely of "FB" shape, and three broad-podded plants with large seed of the characteristic "cylinder-section" type seen in "LM".

The factor for seed shape is therefore very tightly linked to, but not identical with, the major factor for pod breadth.

A marked correlation between narrow pod and high percentage of double racemes was evident in F_2. The relationship is shown in Graph 7. The curves for individual families (omitted from the diagram), though irregular owing to small numbers of plants, all agree with that for the combined F_2 in showing a fall in percentage of "doubles" with increased pod breadth, except in the case of the very narrowest of pods, where there is a general tendency for the converse relationship to hold.

That the association is due to linkage of genes, and not to a physiological reduction in pod breadth directly caused by the added nutritional burden of a second pod on the raceme, is indicated by the absence of the correlation in "FB". (A larger number of plants would, in all probability, have smoothed this curve into one showing a steady mean percentage of two-flowered racemes, unaffected by the (non-genetic) fluctuation of pod breadth.)

Parents of the F_3 were selected for (amongst other features) high percentage of double racemes, narrow and broad-podded alike.

The total BB F_3 (14 families) contained 10 per cent. "doubles".
,, ,, Bb ,, (20 ,,) ,, 9 ,, ,, ,,
,, ,, bb ,, (14 ,,) ,, 29 ,, ,, ,,

The first thing to notice is the seasonal effect, the percentage of "doubles" was very much lower than in 1929 (the decrease was due to some extent to the recording of the whole plant, rather than the main stem only (see page 161).

Nevertheless the efficacy of selection for "doubleness" is shown by comparing the figure 10·1 per cent. for homozygous broad-podded families, with an estimate of 1-2 per cent. "doubles" on "FB" in the same season. It seems to have been less effective in the case of the bb families, from which one concludes that "LM" was already carrying not only the main factor for "doubleness" but all or nearly all the minor ones as well.

The linkage between "doubleness" and narrow pod was still well marked, in spite of the selection; the percentage of twin-flowered racemes for bb plants being nearly three times as great as that for BB plants. (Notice also the relative order of "doubleness" of broad and narrow lines in Table IX.)

The low percentage for the segregating families was surprising. Since comparative "doubleness" was more or less dominant in F_1 and F_2, these families would have been expected to show about 24 per cent. double racemes, instead of which they have rather less than the pure "broads". Fluctuations of sampling of the F_2 parents are unlikely to account for more than a small portion of this deviation from the expected fairly high "doubles" percentage. It is possible or even probable that in an unfavourable year such as 1930 high "doubleness" may behave as a recessive rather than as a dominant character. Even then the Bb families, containing about 25 per cent. of the bb plants, would be expected to average about 15 per cent. twin-flowered racemes!

Assuming the approximate correctness of $33\frac{1}{3}$ per cent. "doubles" as a dividing line between dd, and DD and Dd plants in F_2, the four classes

bbdd	12 plants
BBdd and Bbdd	82 plants
bbDD and bbDd	68 plants
BBDD, BbDD, BBDd and BbDd ..	164 plants

indicate a cross-over value of approximately 28·3 per cent. for the linkage between narrow pod and high percentage double racemes.

The F_2 also showed that a late FF date and broad pod were associated. The correlation, though much smaller than that for seed weight and pod breadth, is definitely significant.

TABLE XVI.

Correlation of pod breadth and FF date (F_2 of X.1 omitted).

Date of First Flower.	Pod Breadth to Nearest Millimetre. 13	14	15	16	17	18	19	20	21	22	23	24	Total.
12/6	—	—	4	2	—	2	1	2	—	—	—	—	11
15/6	1	—	4	3	5	5	3	6	—	—	—	—	27
18/6	—	1	11	19	18	20	17	22	6	3	—	—	117
21/6	—	1	5	12	4	16	16	14	5	2	—	2	77
24/6	—	—	—	7	4	8	9	7	1	1	—	—	37
Totals ..	1	2	24	43	31	51	46	51	12	6	—	2	269

Mean Breadth =18·07 mm. ±1·87.
Mean FF. =18·38 (=19·12/6) intervals ±·98.
Correlation Coefficient =−0·187±·059.

See also Graph 8. The curve for "FB", genetically pure for broad pod, slopes the opposite way to that for the segregating hybrids; i.e. later flowers tend to produce narrower pods. This is perhaps because less vigorous and definitely unhealthy plants are inclined to be later flowering. This effect must be present in the hybrids, but is completely over-ridden by the correlation of genetically late FF with broad pod.

The parent plants of the partial F_3 were frequently selected on the score of earliness, so that the resulting BB and bb families cannot be used to verify the extent of the correlation.

As a matter of fact the average time taken from sowing to FF by the BB F_2 parent plants was 70·3 days, and by the bb plants 72·1 days, yet the means for the respective F_3 generations raised therefrom were 56·24 ± 3·90 and 55·90 ± 3·44 days, with a ("insignificant") difference of 0·44 days. This "reversion" towards comparative lateness on the part of the BB plants can only mean that many of the selected "early broads" owed their earliness to fluctuation rather than to the possession of appropriate genes.

Uncertainty as to the factorial basis of earliness and lateness of FF prevents any attempt at a formal calculation of linkage values.

The only previously reported linkage of time of flowering is one with flower colour (6, 8, 12). (Apart from node number.)

Development after flowering also tends to take longer for broad pods.

This could not be seen from the F_2 data since the scoring interval for FP (first pickable pod) date was sometimes as much as seven days, making the records useless.

The F_3 was labelled at two day intervals, as for FF date. It was practically impossible to exercise conscious selection of short FF-FP interval on the F_2; and any selection of earliness as a whole, if it affected this point, would act in the direction of reducing the period of pod development to an extent greater for BB than bb families.

The difference between the mean FF-FP intervals for BB and bb plants given below should therefore be increased, if anything, to be truly representative.

TABLE XVII.

Distribution of FP-FF interval in F_3 plants.

Plants.	FP–FF interval in days.								Mean.	S.E. of Mean.
	26	24	22	20	18	16	14	12		
BB ..	3	35	53	78	69	12	2	—	20·26 ± 2·36	·15
bb ..	—	3	14	88	207	92	37	2	17·79 ± 1·96	·09

Difference between means for BB and bb plants = 2·47 days, ± ·24.

The difference between BB and bb plants of this generation is not so large as that between "FB" and "LM" (page 164), which may mean that linkage is not complete, or that modifying factors are at work; or just possibly that 1930 favoured development to a greater extent in broad pods than in narrow.

Individual F_3 families were frequently too small to show statistically significant differences, especially after diseased or broken plants had been discarded. The following table gives figures for a few pure breeding (for pod breadth etc.) lines selected at random. It illustrates the general tendency for FP-FF interval to be shorter for narrow-podded lines. Within the BB and bb groups at least the bracketed pairs show significant differences in mean FP-FF interval, so that linkage of this character with the main pod breadth factor cannot be absolute.

TABLE XVIII.

Lines differing in FP-FF interval.

F_3 Family.	Pod.	No. of Plants.	Mean FP—FF Interval in Days.	Standard Error of Mean.
7.4.23 ..	bb	46	16·87 ± 2·45	0·36 ⎫
7.3.9 ..	bb	20	19·2 ± 1·55	0·35 ⎭
5.1.2 ..	bb	21	17·0 ± 2·37	0·58
6.5.10 ..	bb	50	17·76 ± 1·76	0·25
1.1.23 ..	BB	13	20·9 ± 2·24	0·62
3.4.2 ..	BB	15	18·8 ± 1·54	0·40
2.1.9 ..	BB	15	22·1 ± 1·55	0·40 ⎫
7.3.1 ..	BB	25	18·2 ± 2·33	0·45 ⎭

In 1932 some lines had broad pods taking appreciably less time to develop than those of "FB". The correlation of broad pod with long FF-FP interval is therefore not merely an automatic result of the greater nutritional requirements of a larger pod.

It was mentioned in the Introduction that "LM" flowers at a less height from the ground than "FB".

Height to the first raceme is a compound character, depending on both length and number of sterile internodes, and as a detailed study of these points has not been undertaken no attempt has been made to elucidate its genetical behaviour in this cross.

It is nevertheless interesting to note that the "LM" features of low flowering and early flowering are associated in the hybrids (*cf.* Keeble and Pellew (7), Hoshino (6)).

TABLE XIX.

Correlation of height and date of FF.

Date of FF.	Height to First Raceme, to nearest inch. 10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	No. of Plants. Totals.
12/6	3	1	5	—	1	1	—	—	—	—	—	—	—	—	—	11
15/6	6	2	9	1	5	1	3	—	1	—	—	—	—	—	—	28
18/6	1	2	4	6	14	19	18	18	19	4	3	—	—	—	—	108
21/6	1	—	1	3	6	9	9	16	8	7	5	4	1	—	—	70
24/6	—	1	1	1	3	2	5	6	6	3	3	2	—	—	1	34
Totals	11	6	20	11	29	32	35	40	34	14	11	6	1	—	1	251

Mean height to first fertile node $=15\cdot76\pm2\cdot70$.
Mean date of first flower $=19\cdot06/6\pm2\cdot94$ days ($\cdot98$ intervals).
Correlation Coefficient $=\cdot51\pm\cdot047$.

Foliage colour alone segregates independently of the remaining complex of characters investigated.

TABLE XX.

Pod breadth of pale and dark foliaged plants.

Foliage Colour	Pod breadth to nearest millimetre. 13	14	15	16	17	18	19	20	21	22	23	24
Dark ..	1	3	19	35	29	49	39	46	16	6	—	—
Pale ..	—	1	5	13	9	10	11	21	—	1	—	2
Per cent. Pale	—	25·0	26·3	27·0	23·7	17·0	22·0	31·3	0·0	14·1	—	100·0

In terms of "broad" and "narrow" classes, the above distribution is:

		Theoretical, assuming free segregation.	*Difference.*
Dark "broad" ..	185	177·75	+7·25
Dark "narrow" ..	58	59·25	−1·25
Pale "broad" ..	54	59·25	−5·25
Pale "narrow" ..	19	19·75	−0·75

the actual numbers being a good approximation to those theoretically required by the absence of linkage.

DISCUSSION.

Two features of the results obtained in this investigation are outstanding. Four of the six characters dealt with, i.e.

foliage colour,
pod breadth,
seed weight,
and percentage of double-flowered racemes,

are each shown to be determined, in the main, in the material used, by a single pair of allelomorphs ; but in every case minor or modifying factors must be responsible for smaller variations within the major classes. This is probably true of seed shape also, but is there more difficult to demonstrate ; also the complicated behaviour of FF data provides ample room for the action of such supplementary determinants.

"LM" and "FB" are very far from representing the extreme range of variation of the garden pea in any of the above features. Broader, and far narower-podded varieties occur in innumerable steps ; slightly larger and much smaller seeded forms, earlier and later flowering, and less twin-bloomed types. What is the genetic basis of the extensive series of modifications of any single character ?

It has been shown that the simplest relationship exists between "LM" and "FB" foliage colour, but that a "very dark" occurs, and also that under certain conditions "pale" can be subdivided into more and less pale. The much more pronounced difference between "green" (which particular shade was worked with one does not know) and "yellow green" apparently depends on a single allelomorphic pair (27). Is the expression of the "green" factor modified by the action of another pair of genes present in "LM" and "FB" ? Or are the shades found in those varieties dependent on alternative "green" allelomorphs situated at the same locus ?

The minor variations found in the hybrid progeny of this cross must be due to modifying factors, unless further mutations have taken place.

The Tedin's (17) found, for node number, a similar state of affairs ; a main 3 : 1 segregation, with pure lines for many intermediate numbers nevertheless existing. They favoured a multiple factor hypothesis.

Wellensick's review (25) of the literature shows that in pea crosses F_1's are usually somewhere between the parents in character, and transgression of the parental limits in later generations rare, whatever feature is being considered. (Tedin (16) did find transgressive inheritance of flowering date. See page 169.)

This suggests that major factors for any character are usually situated at one locus only ; otherwise it would be expected that the meeting of two dominant genes in F_1 and later segregates would result in transgression of the parental values. Keeble and Pellew's F_1 (7) was taller than either parent, owing to such a meeting of dominants, height being a compound character depending on both internode length and number.

It seems probable that both multiple modifying factors and multiple allelomorphs (such as have indeed been shown to determine leaf axil colour by the Tedins (18) in a later paper), are concerned with the determinations of quantitative characters in peas.

It is of interest that all the features investigated, except foliage colour, should be correlated together as in the parental forms.

The haploid chromosome number of Pisum is seven.

That of seven genes or groups of genes (eight if height to FF node is included) selected for study simply because of their practical importance, all but one should chance to be located in the same chromosome is very unlikely.

In addition, observation shows that the lower disease resistance (especially to mosaic), higher percentage of undeveloped ovules, more pod abortion, greater susceptibility to environmental influences generally, and more branching habit, characteristic of "FB", all tend to be associated with broad pod, low percentage of paired flowers and other "FB" features in the hybrids.

The occurrence of segmental interchange, and ring formation, in the chromosome complement of Pisum has been described in various races by Richardson (13), Hammarlund and Hakansson (4), Pellew and Sansome (12) and Sansome (14), so that it is quite possible that chromosomal linkage may be responsible for this effect. It is doubtful if any great percentage of gametic sterility occurs in the material used here, but partial sterility is not an inevitable accompaniment of catenation. Cytological observation should speedily decide if rings are formed in "FB" or "LM".

The observed correlations are to some extent such as might be expected on physiological grounds. That a heavy-seeded broad foliaged and broad-podded plant should take longer to develop than a lighter-fruited one is understandable; similarly it seems reasonable to find that the larger pod is less frequently paired. Again, the longer the stem grows before flowering the later the flower may be expected to be, and that larger seed of less compressed shape should be present in the bigger and proportionally roomier pods is natural. Yet the fact that early maturing broad-podded, and late narrow-podded lines; broad-podded races with a high percentage of twinned flowers, and plants with large seed of "LM" shape, have been isolated, demonstrates that genetically speaking different, though usually linked (in some way or other) determinants are concerned. It is nevertheless true that some, at least, of these "crossover" combinations appear to be physiologically less well balanced than the parental types. Many lines of earlies with broad pods have been selected as of possible economic value, but most of them have proved to be unusually feeble and susceptible to disease. This is an instance of the partial practical failure of one of the theoretically desirable types, the production of which was the main object of the original hybridization.

"Little Marvel", at any rate, is one of the older varieties of wrinkled garden pea, and is still very widely grown. Its success may be due in no small measure to an excellent physiological balance: and its persistence, through the vicissitudes of commercial seed growing and "improvement", to the strongly linked hereditary behaviour demonstrated here for some of its outstanding characteristics.

Hedrick (5) follows practical opinion in repeatedly asseverating the greater stability, over a period of years, of some varieties of pea than of others. The more dogged survival of certain "fundamental types" would be explained if such marked association of characters found here is at all general in garden peas. The fact that a vast literature on the genetics of Pisum makes no mention of such a phenomenon (with the exceptions quoted (13), (14), (12), (4)) is not conclusive evidence against this possibility, since the great bulk of previous work is concerned with easily scored major variation, such as purple flower colour, present in forms which lie to a great extent outside the province of the practical grower and breeder.

The other point of general practical interest is a confirmation of the importance of selection, even within what a cursory investigation would dismiss as a pure type. Unfortunately it is frequently impossible to distinguish genetically determined variation from mere fluctuation except by one or more progeny tests. Nevertheless the number of factors controlling, say, seed weight in peas, available for selection, must be great, although their individual effect is frequently correspondingly small. Fisher (3) has recently pointed out how the early "Mendelians" were at fault in condemning the practical man's belief in the efficacy of selecting small variations.

SUMMARY.

The F_2 and partial later generations, of a cross between two varieties of garden pea are examined with regard to certain characters contrasted in the parents.

Pod Breadth. A mainly simple relationship is found to hold, modified by minor genes, or possibly by factorial inter-relationships in some cases.

Seed Size is very difficult to work with, owing to its great susceptibility to environmental influences, and especially to varying severity of attack by virus disease. It is probably essentially dependent on a single pair of allelomorphs.

Seed Shape is probably determined in a similar manner to seed size.

Percentage of Two-flowered Racemes. This also is subject to abnormally great fluctuation, but a general tendency for a high percentage to dominate over a low one exists, and there is some evidence for a major monohybrid segregation. In addition, the possibility of isolating a series of lines characterized by different relative proportions of two-flowered racemes is demonstrated.

Period. The time required for the first pod to be fit for culinary use, from seed sowing, is subdivided into two portions ; from sowing to the opening of the first flower, and from then until the resulting pod is ready to pick.

In this cross earliness of flower tends to be recessive, but the relationship of "LM" period to "FB" period is complex. The results obtained by other workers are discussed.

The period required for pod development differs too little in the respective parent strains, and was too inexactly scored in F_2, for its mode of inheritance to be susceptible of analysis.

Foliage Colour. It is shown that a slight difference in depth of green behaves as if determined by a single allelomorphic pair of genes. Some further modification may occur.

Correlation of Characters. Narrow pod, small seed, "LM" shaped seed, many two-flowered racemes, early flowering, rapid pod development, and short stem to the first fertile node are shown to be associated, to a varying degree, in the hybrids. From field observation, resistance to disease and insect attack, capacity for withstanding adverse conditions and ability to develop nearly all ovules with constancy, might be added to the above list.

That is, all the examined characters, except foliage colour, tend to remain associated together in the parental combinations. At least some of these features might be expected to be physiologically interdependent, but the stabilizing of lines showing new combinations shows that some form of gene linkage is responsible for many or all of the correlations.

The genetic complexity which must exist for quantitative characters in peas is pointed out, and the possible theoretical and practical significance of the very comprehensive "linkage" found is briefly discussed.

REFERENCES.

(1) *Bateson, W.* and *Pellew, C.* On the Genetics of "Rogues" among Culinary Peas (*Pisum sativum*). Journ. Gen., 5, p. 13. 1915.

(2) *Denaiffe, —.* Les Pois Potagers, Paris. J. B. Bailliere et Fils. 2 eme. impr., 288 pp. 1906.

(3) *Fisher, R. A.* Number of Mendelian Factors in Quantitative Inheritance. Nature, 131, p. 400. 1933.

(4) *Hammarlund, C.* and *Hakansson, A.* Parallelism of Chromosome Ring Formation, Sterility and Linkage in *Pisum*. Hereditas, 14, p. 97. 1930.

(5) *Hedrick, U. P.* Vegetables of New York, 1, part 1: Peas. Rept. year ending June 30th, 1929, N.Y. State Agri. Expt. Sta., Geneva, New York. 1929.

(6) *Hoshino, Y.* On the Inheritance of the Flowering-time in Peas and Rice. Journ. Coll. Agri. Tohoku Imp. Univ. Sapporo, 6, p. 229. 1915.

(7) *Keeble, F.* and *Pellew, C.* The Mode of Inheritance of Stature and of Time of Flowering in Peas (*Pisum sativum*). Journ. Gen., 1, p. 47. 1910.

(8) *Lock, R. H.* Studies in Plant Breeding in the Tropics, I. Ann. R. Bot. Gard., Peradeniya, 2, p. 310. 1904.

(9) ———. Studies in Plant Breeding in the Tropics, II. Ann. R. Bot. Gard., Peradeniya, 2, p. 357. 1905.

(10) ———. Present State of Knowledge of Heredity in Pisum. Ann. R. Bot. Gard., Peradeniya, 4, p. 93. 1907.

(11) *Mendel, G.* Versuche uber Pflanzenhybriden. Verh. naturf. Ver. Brunn, 4, p. 3. 1865.

(12) *Pellew, C.* and *Sansome, E. R.* Genetical and Cytological Studies on the Relations between Asiatic and European Varieties of *Pisum sativum*. Journ. Gen., 25, p. 25. 1931.

(13) *Richardson, Eva.* A Chromosome Ring in *Pisum*. Nature, 124, p. 578. 1929.

(14) *Sansome, E. R.* Segmental Interchange in *Pisum sativum*. Cytologia, 3, p. 200. 1932.

(15) *Tedin, H.* Några synpunkter vid förädling af ärter. Sver. Uts. för. Tidskr., 7, p. 111. 1897.

(16) ———. Växförädling. Ref. in: Zach. f. Pfl. Züchtung, 3, p. 254. 1915.

(17) *Tedin, H.* and *Tedin, O.* Contributions to the Genetics of *Pisum*. III: Internode length, stem thickness and place of the first flower. Hereditas, 4, p. 351. 1923.

(18) ———. Contributions to the Genetics of *Pisum*. IV. Hereditas, 7, p. 102. 1925.

(19) *Teräsvuori, K.* Über in Finnland feldmässig gebaute Erbsenformen. Acta Societatis pro Fauna et Flora Fennica, 40, No. 9, 142 pp. 1915.

(20) *Tschermak, E. von.* Ueber die gesetzmässige Gestaltungsweise der Mischlinge. Zsch. landw. Vers. Wesen Oesterreich, 5, p. 781. 1902.

(21) ———. Ueber die Vererbung der Blütezeit bei Erbsen. Verh. naturf. Ver. Brünn, 49, p. 169. 1910.

(22) ———. Bastardierungsversuche an Levkojen, Erbsen und Bohnen mit Rüchsicht auf die Faktorenlehre. Zsch. ind. Abst. u. Vererb. L., 7, p. 81 (see pp. 135-82). 1912.

(23) ———. Über Züchtung landwirtschaftlich und gartnerich wichtiger Hülsenfruchten. Arb. D.L.G. für Oesterreich, 4, p. 80 (see pp. 85-6). 1919.

(24) *Ubisch, G. v.* Beitrag zu einer Faktorenanalyse der Gerste. Zeitsch. f. induktive Abst. und Vererbungslehre, 25, p. 198. 1921.

(25) *Wellensiek, S. J.* Genetic Monograph on *Pisum.* Bibliographia Genetica, 2, p. 343. 1925.

(26) ———. *Pisum*—Crosses. I. Genetica, 7, p. 1. 1925.

(27) *White, O. E.* Studies of Inheritance in *Pisum.* II: The present State of Knowledge of Heredity and Variation in Peas. Proc. Amer. Phil. Soc., 56, p. 487. 1917.

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ROOT STUDIES

VI. APPLE ROOTS UNDER IRRIGATED CONDITIONS, WITH NOTES ON USE OF A SOIL MOISTURE METER

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THE Okanagan Valley in British Columbia offers some very interesting comparisons with the fruit areas of England. The greatest contrast is provided by the fact that the Okanagan Valley lies in the dry belt between the Rocky Mountains and the Coast Range, and has an annual rainfall of about 9 inches. Consequently the orchards in this area depend on artificial irrigation for their moisture supply.

The present writer was fortunate to have the opportunity to make some root studies under these special conditions during the season 1931-2 when he was stationed at the Dominion Experimental Station, Summerland, British Columbia, under a scheme of exchange of research workers within the Empire, arranged by the Empire Marketing Board.

PART I.

APPLE ROOTS UNDER IRRIGATED CONDITIONS.

Material.

This series of root studies included the excavation of the complete root system of an eight-year-old McIntosh apple tree, and the excavation in less detail of 175 three-year-old apple trees of various varieties and the main part of the root system of a twelve-year-old Winesap. The latter was suffering from Collar Rot. These trees were all on seedling roots with the exception of 10 three-year-old trees which were on Malling clonal rootstocks Nos. XII and XIII. The object of the studies was to observe the type of root growth given by irrigated conditions—to note the reaction of the seedling and clonal root systems to the various scion varieties worked upon them, and if possible to gain some preliminary information on the action of Collar Rot.

A soil moisture meter was devised and used to show the soil water movements in the orchard beside the eight-year-old McIntosh. A brief description of the instrument and some of the results obtained therefrom are included in this paper.

The amount of work that it was possible to carry out was limited by the time available, and it must be emphasized that the results obtained can only be regarded as preliminary in nature.

A further study of actual period and rate of root growth at Summerland, carried out by means of an observation trench in the orchard, has already been reported on (4).

A. *The roots of an eight-year-old McIntosh apple tree.* This tree was excavated in detail by the trench system described in references (1) and (2). A trench was dug beyond the root area, and worked in half metre " steps " across the root system. The soil was carefully removed from the roots with a small hand fork, and the roots were marked on a plan and weighed in 50 cm. square blocks in 4 depths.

The soil in the irrigated orchard was of a fine sandy nature, overlying sand at 1 metre* and gravel at 1·2 metres. The orchard was cover-cropped with alfalfa (lucerne) whose strong tap roots penetrated to 150 cm. and more. One of these alfalfa tap roots is shown in Fig. 1, which also shows the soil profile.

Branch and Root Spread.

The vigorous growth obtained under the conditions of hot sunshine and artificial irrigation is well shown by the branch measurements of this eight-year-old tree. The height was 5·4 m. (17 ft.), the mean branch spread 3·55 m. and the girth 31·5 cm. at 30 cm. from ground level. Fig. 2 shows the tree, the excavation trench at an early stage, and the many small roots found at that distance. The mean root spread was 8 m. (25 ft.), i.e. over twice the branch spread. Roots from neighbouring trees 9·2 metres away (sixteen years old) were found at distances of 6 m. and 7 m. from their trunks. The very numerous and fine roots of this tree made the excavation the most difficult yet undertaken by the writer.

Type and Position of Roots.

Fig. 3 shows the trench nearer the tree. It can be seen that most of the roots are in the top 50 cm. of soil, but one of the roots that curved downwards is shown up by the white paper. In general the roots kept in the top metre of soil, though one or two roots penetrated to a maximum of 146 cm. in the moist gravel. The beginning of the white sand can be seen to the left of the trench in Fig. 3.

Fig. 3 also shows the technique of root dyeing used in this excavation. The trunk of the tree was cut off and a 1 ft. length of motor tyre tubing (including the valve) was slipped on to the stump and bound with string. Some " light green " dye was then poured into the tubing, and the top end was blocked. A small pressure was then applied by inflating with a pump. Sap was observed exuding from the cut ends of roots three hours later, and six hours later the green dye had reached the cut ends. This was a great help in sorting out the roots from overlapping roots of other trees. The test was not absolute, since the dye did not penetrate far in uncut roots, but once the centre of the tree was passed this did not matter, for the excavators were following known and labelled roots.*

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* 1 m. = 3·28 ft.

On reaching the centre of the tree a mass of small roots was found, as shown in Figs. 4 and 5. There were seventy-seven roots of about 1 cm. diam. surrounding the trunk. An upper whorl of roots had eight roots 2 to 3 cm. diam. It is possible that these latter were scion roots, though this cannot be stated with certainty.

There were no tap roots below this tree. What appeared to be the original seedling root dwindled rapidly from 5·5 cm. diam. at the trunk to three branches of 2·8, 1·6 and 3·4 cm. diam., which did not penetrate far vertically downwards. Only one root below 50 cm. was more than 1·5 cm. in diameter.

The large fangy roots normally associated with seedling root systems were certainly not present in this case. The next tree, however (sixteen years old), when partly excavated for the placing of an observation trench, showed several rather more deeply descending main roots.

In general the root system strongly resembled two trees of Lane's Prince Albert on the vigorous stock O.F.5 excavated from a light sand at Wisley, Surrey (2). In each case the roots were very numerous and wiry and had a comparatively small amount of fine fibre.

The two soils were very similar in texture. Certain striking contrasts in root amount and position are therefore of some interest. It must be noted that the comparison is by no means exact, since both variety and rootstock are different, but this is the best comparison at present available, and certainly gives food for thought.

Branch and Root Weight.

The total branch weight of the McIntosh tree was 37·1 kilograms (82 lbs.) and the total root weight was 18·6 kg. Thus the ratio of branch to root (in the dormant state, March, 1932) was 1·99 kg. of branch per kg. of root. This compares with 0·72 kg. branch per kg. root of the ten-year-old Lane's Prince Albert apples on sand mentioned above.

This large contrast possibly gives some indication of the increase in "efficiency" of the root system caused by artificial irrigation combined with other climatic factors of the Okanagan. It may also be noted that the ratio of root spread to branch spread was 2·3 for the irrigated tree and 2·8 for the tree at Wisley.

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* A more satisfactory injection technique has now been devised by Mr. W. A. Roach, of East Malling Research Station. He finds that if the trunk of the tree is cut off and dye is applied about a fortnight before excavation, practically every root becomes dyed. See Roach, W. A., "Injection for the diagnosis and cure of physiological disorders of fruit trees." *Journ. of Applied Biology*, 1934, XXI, 333-43.

Depth of Root.

The percentage of root of the irrigated McIntosh at different depths was as follows :

Depth.		Percentage of total root weight.
0 to 30 cm.	:	29·6 per cent.
30 to 50 cm.	:	48·9 ,,
50 to 100 cm.	:	20·9 ,,
Below 100 cm.	:	0·6 ,,

This shows clearly how the concentration of roots was greatest between 30 and 50 cm. deep, or roughly 1 ft. to 1 ft. 8 in. This is probably the combined result of the surface irrigation and cover cropping.

It would appear likely that the roots of the cover crop took precedence in the top 30 cm., while the irrigation water would usually only wet the soil to a depth of 4 to 5 ft. (See Part II of this paper.)

By way of contrast the apple trees at Wisley had 76·4 per cent. of their root weight above 30 cm.

On the sandy soil at Summerland there was no indication that roots had been killed by lack of aeration due to too much water. On clay soils not far away it was clear that over-irrigation could seriously restrict the root system in this way. This depth-limiting factor of lack of aeration was also noted in clay land excavations in England (2 and 3).

B. YOUNG APPLE TREES.

Seedling apple roots have been used almost exclusively as stocks for apples in the Okanagan. It is now recognized that the great genetic variation in these seedlings may be responsible for some of the rather large annual loss of trees from orchards of all ages. Careful selection of the seedlings often results in tree growth of remarkable uniformity, but it is only to be expected, since the root stocks are of different genetic make-up, that their resistance to various troubles caused by the climatic extremes of the Okanagan should vary greatly. In the experimental nursery at Summerland it was noted that individuals in the rows of seedling apples showed great variation in leaf characteristics and time of leafing out, even when raised from pips of the same variety. An orchard of mature seedling trees of known parentage (crossed to raise new varieties) showed even more striking contrasts in season, growth and fruiting—as might be expected. To observe the behaviour of seedling roots worked with known varieties, 175 three-year-old apple trees of fourteen varieties worked on Canada Baldwin seedling apple roots were excavated in some detail. Since the trees were to be replanted no root or fibre analysis could be made, but careful notes were made of the root systems, and photographs were obtained. Some trees of the same age but worked on Malling clonal stocks were excavated for comparison. The varieties were : McIntosh Red, Jonathan, Wealthy, Newtown

Pippin, Black Twig, Winesap, Turner Red, Hyslop, Melba, Northern Spy, Delicious, Rome Beauty, Canada Baldwin and Stock 227.

It was found that even when the tops were fairly uniform the seedling roots, in spite of careful grading at the time of grafting, showed much variation. They could fairly readily be divided into shallow, medium and deep rooting types, no matter what variety was worked on them. On the whole the strong growing trees had big root systems, and the weak growing trees had smaller root systems, but in this experiment there was certainly no readily visible qualitative difference between the seedling roots worked with these different varieties when they were laid out in a row after excavation.

Ten trees of the variety McIntosh Red on Malling clonal stocks Nos. XII and XIII were excavated for comparison with the same variety on seedling stocks. There was a great contrast between the clonal and seedling rootstocks. The clonal rootstocks under these conditions had numerous small roots just as they do at Malling at that age, while the seedling stocks had fewer roots with a much more marked downward tendency. The clonal stocks had been transplanted after their first year, while the seedling stocks had not, but this could hardly account for the great contrast. In any case, it was evident that the variety (McIntosh) that was used on both seedlings and clonal stocks, had not exercised a predominating influence in the growth of the roots, since there was such a great difference between the trees on the seedling roots and those on clonal roots.

Both roots and branches showed remarkable vigour under these irrigated conditions. Some roots reached a length of 5 ft. and a depth of 3 ft. 6 in. in the two years after transplanting.

Scion Rooting.

It was noted that many of the young trees mentioned above had formed small adventitious roots above the union, i.e. scion roots. Out of fourteen varieties excavated, nine showed definite scion roots at this early age. Some examples are given below.

Variety.	No. of Trees.	No. of Trees with Scion Roots.	Percentage with Scion Roots.
Rome Beauty	17	17	100
McIntosh Red	12	12	100
Hyslop Crab	8	8	100
Stock 227	15	8	53
Turner Red	5	2	40
Melba	16	6	38
Delicious	14	4	29
Northern Spy	15	0	0

Care must be taken to distinguish between scion roots and a second whorl of roots that often arises from the stock above the original roots. It should be noted also that at this age the amount of scion root is very small. There is no doubt however that where the base of the scion is buried—as is done with these root grafts—trees that have a tendency to form scion roots may become established on their own roots to a greater or less extent in the course of time, so adding a further uncertain factor to the already unknown capabilities of the seedling root system. This danger can be avoided by high working—a procedure easy to carry out with vegetatively raised stocks, but almost impossible with the root grafting technique used for seedling stocks.

C. Collar Rot.

One of the most serious sources of tree loss in the Okanagan is from "Collar Rot", a trouble which causes the tree to weaken and die, sometimes in a single season, and when in full bearing. The leaves become small and bronzed, the fruit fails to swell and the whole branch system shows severe starvation symptoms (Fig. 6). References are made to this trouble in reports of the Dominion Botanist (1928, 1930, etc.), but little certain information on the cause yet seems available.

A Winesap tree about twelve years old suffering from this trouble was excavated by the writer in June 1932. It was found that most of the main roots were dead at the crown, and in some cases were dead throughout and rotting away, as shown by the roots marked A in Fig. 7. In other cases the lower portion was still living, as shown by the suckers arising at the end of the live portion (B, Fig. 7). In this tree a few roots were still alive throughout. It seems clear that in this case the branches were being cut off from their root food and moisture supply and were starving to death. The roots that were continuously deprived of any carbohydrate supply would also naturally die.

Whether frost injury or some organism or other cause is responsible for this crown rot, it appears likely that root stocks showing greater resistance to this trouble could be found, and could be used with beneficial effect.

It is not suggested that this preliminary work does more than show the possibilities of further work on these lines. It is quite clear, however, that many of the problems of irrigated fruit in the Pacific North-West can profitably be tackled at the root.

PART II.

Notes on the Use of a Soil Moisture Meter.

A full appreciation of the relation of root growth to soil conditions, especially under artificial irrigation, can only be gained by taking into account the actual moisture movements in the soil. The standard method of estimating soil moisture by taking a soil sample and drying it proved too slow and laborious for extended use, and consequently the continuous reading moisture meter described below was devised. This meter was very useful in showing where the irrigation water was getting to and how it was used up by the tree roots. The particular meters described below were installed beside a root observation trench, in which the root growth could be observed and its relation to the soil moisture could be noted. The observation trench was 30 ft. away from the excavation site of the eight-year-old McIntosh apple described earlier in this Report.

While the records given below apply to this particular area, it will be seen that the instrument may be of value in a much wider field, since a reliable indication of the soil moisture movements is just what every irrigator requires in order to apply the water in the most efficient manner.

Construction of the Moisture Meter.

This instrument depends on the "capillary pull" due to the surface tension of the film of water surrounding the soil particles. This force varies with (*a*) the degree of fineness, (*b*) the compactness and (*c*) the moisture content of the soil. Factors (*a*) and (*b*) remain constant in any one position, hence by measuring the capillary pull the soil moisture can be estimated. The actual capillary pull is also a valuable figure to have, since this is probably the nearest single measure of the resistance of the soil to water absorption by the plant at present available. This instrument measures the capillary pull by means of a porous pot filled with water, buried in the soil at the required depth, and connected by a copper tube to a mercury manometer or a vacuum gauge. The soil draws water out of the pot until the manometer height corresponds to the capillary pull of the soil. For success as a field instrument several modifications are necessary. (1) a thick walled dense grained porous pot adapted for airtight connection to a copper tube, (2) a device to prevent air dissolved in the soil water from entering the manometer tube, (3) a device to prevent freezing in winter, (4) a device for periodical re-filling of the porous pots.

Point (1) is obtained by using specially made pots with a neck suitable for connection to a brass bush. Points (2) and (3) are gained by fixing a small rubber balloon at the base of the main tube, inside the porous pot, and having the main tube and rubber sac filled with glycerine solution. Point (4) is gained by fitting two filling tubes with airtight stoppers to the brass bush so that water can be added through one tube while air is let out of the other.

The manometer in the field model is held in a sloping wooden case. The slope magnifies the mercury movements, and makes reading easier (Fig. 8). A full description of these instruments with particulars of laboratory tests and results in orchard land and fallow ground has just been published (5). Messrs. A. Gallenkamp, Sun Street, London, E.C.2, are now manufacturing both a laboratory and a commercial form of the instrument. In the commercial form the manometer is replaced by a Bourdon tube vacuum gauge.

Relation of Capillary Pull to Moisture Content.

At saturation the pull is nil. The drier the soil the greater the pull. The pull at a given moisture content (above saturation) is greater for a fine grained soil than for a coarse grained soil, hence to read actual moisture percentage in a given position calibration is required. Calibration curves for the Summerland orchard are given in Fig. 9. It will be seen that the coarse sand at 3 ft. gives a lower pull at a given moisture content than the fine sandy soil at 1 ft. The large natural variation between the moisture content in different spots at one time (combined with the unavoidable errors of sampling) is shown by the variation between the sample borings taken at the same time (in the same horizontal line in the graph). Hence it is clear that any single figure for moisture content of a large area can only be an approximation.

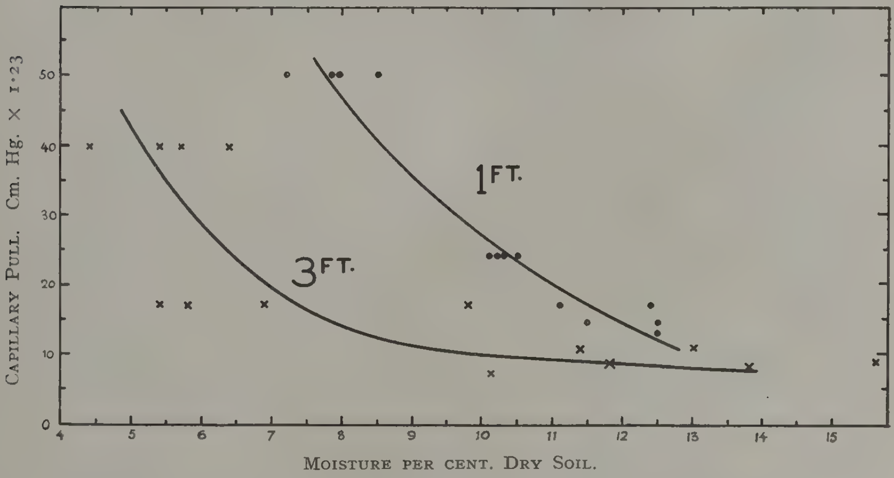


FIG. 9.

Relation between soil moisture and capillary pull in light sandy soil at Summerland, B.C. Dots show moisture content of replicate soil samples at 1 ft. taken near the meter. Crosses show moisture content of samples at 3 ft.

Range.

The range of the instrument is limited to the amounts of moisture giving a capillary pull of up to about 65 cm. of mercury. When the capillary pull exceeds this figure, the water is gradually lost from the porous pot with no increase in manometer reading. Under these conditions the pot can be refilled through the filling tubes as often as is necessary. The actual moisture percentages covered are :—in Sand saturation to about 1·8 per cent. (calculated on dry weight), in Loam, saturation to about 8·0 per cent., and in Clay, saturation to about 22·0 per cent. The working of the instrument is seen in Fig. 10, where the dotted line shows the capillary pull (or " soil dryness ") at 1 ft., and the solid line the capillary pull at 3 ft. in the irrigated orchard. It can be seen from the rising lines that the soil dries almost equally at 1 ft. and 3 ft., since the roots of the tree and cover crop extended throughout the soil to this depth. A rainfall of ·27 inches reached 1 ft., but not 3 ft. Irrigation of 3 inches to 5 inches of water penetrated to 3 ft., and the falling lines on the graph show that the water continues to percolate from the saturated areas round the irrigation furrows to the drier parts of the soil for two or three days. The porous pots were placed 18 inches to one side of the furrow.

When irrigation was withheld for six weeks the soil became very dry and under these conditions an irrigation of 5 inches of water did not reach the meters till two days later, in fact the mercury continued to rise for two days before finally falling. These results support the theory that a given portion of soil must be wetted approximately to its field capacity before it allows an appreciable amount of water to pass on to other portions of the soil. They also show how much more slowly water moves in the soil when the soil is dry than when it is moist.

The relation of soil moisture to root growth as seen in the observation trench has been discussed more fully in another report (4). One of the most important points is the apparent limiting action of the soil moisture under these conditions as shown by Fig. 10. It will be seen that as the soil temperature rises, the root growth, as shown in both number and length of roots, increases also. A fall in temperature is followed by a fall in root growth. When the soil becomes drier, however, the root growth decreases although the temperature is rising (June 27th-30th). When irrigation is applied (June 30th) the root growth rises again sharply. Similar responses are seen throughout the graph. Thus it appears that root growth has varied directly with soil temperature, with sufficient moisture as a limiting factor. The observation trenches at Malling have confirmed this point in two recent drought years. This decrease of root growth owing to moisture deficiency occurred long before the tree showed any sign of leaf wilting.

The actual point at which optimum growth conditions are obtained may well vary with different soils and possibly with different plants. Veihmeyer (6) considered that any moisture percentage above the wilting point was sufficient for good growth of prunes and peaches. Heck (7) suggests that sugar cane grows best at a moisture corresponding to a capillary pull of less than 30 cm. hg. It may well be that the root is *able* to absorb water against a capillary pull of

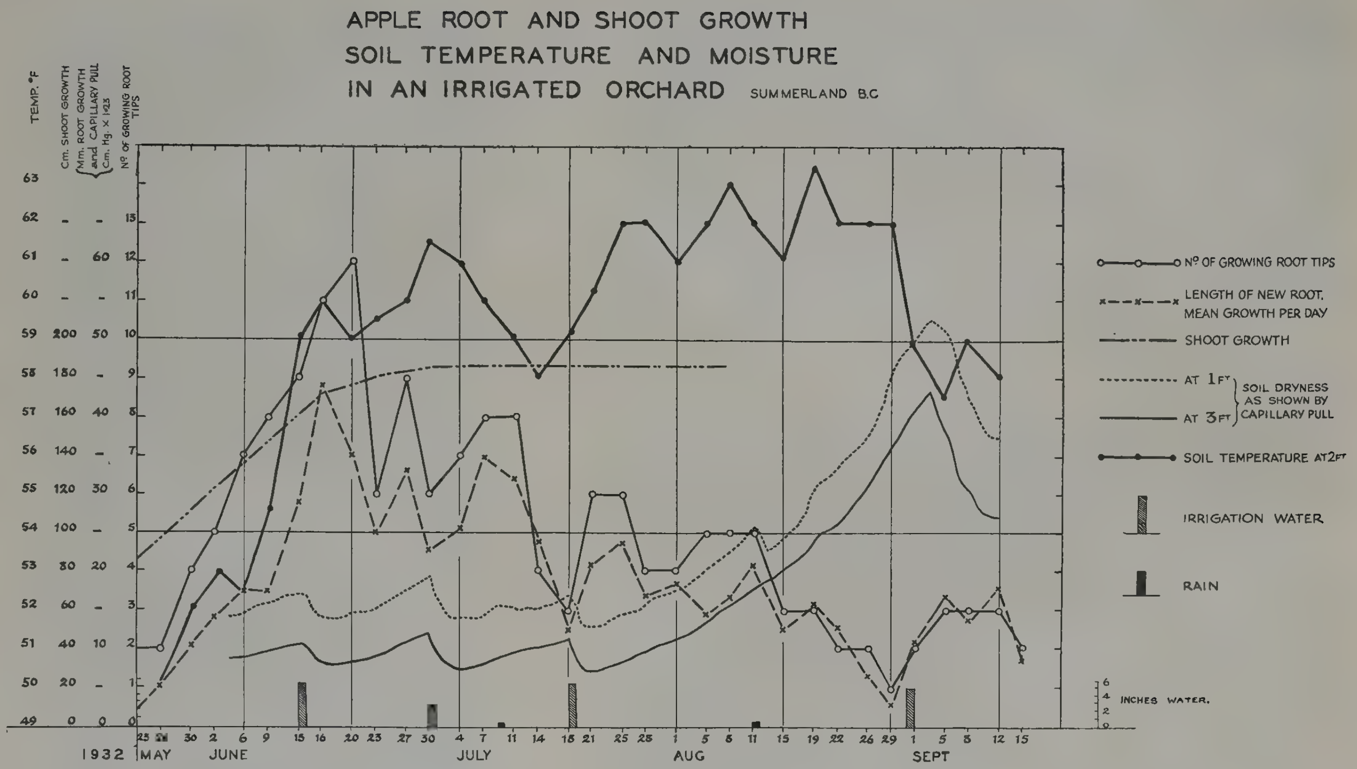


FIG. 10.

Soil moisture and temperature in an irrigated orchard, and their relation to apple root and shoot growth.

several atmospheres, but that when the capillary pull exceeds say 30 cm. hg. in a sandy soil or possibly 50 to 60 cm. in a heavy loam—the water will not move to the root sufficiently fast for its requirements, and a local drought condition may be set up. These points can only be proved by further experiments (which are made practicable by the use of the capillary pull meter).

It appears from these preliminary experiments that optimum root growth of apples on sandy soils or light loams is obtained at a capillary pull below 30 to 40 centimetres of mercury.

Since the above notes were written Dr. Schofield of Rothamsted has published his findings on the suction forces of soils, introducing the scale of measurement which he terms the " pF scale " (*Transactions of the Third International Congress of Soil Science*, 1935, Vol. II, pp. 37-48). The pF is the logarithm of the suction force in centimetres of water. The suction forces of 30 and 60 cm. hg. mentioned above therefore correspond to pF values of approximately 2·6 and 2·9 respectively.

ACKNOWLEDGMENTS.

The writer is greatly indebted to the authorities of the Dominion Experimental Station at Summerland for placing at his disposal the material used in the excavations. He thanks especially Mr. Harold Smith for his valuable help in the excavation and orchard work ; and Mr. E. Fleming, Dr. H. R. McLarty and the whole Summerland staff, who spared no effort to make his visit to British Columbia both instructive and pleasant.

SUMMARY.

1. An eight-year-old McIntosh apple on a seedling rootstock was excavated in detail from an irrigated orchard at Summerland, B.C. The branch height was 5·4 m., the mean branch spread 3·55 m., and the mean root spread 8 m.

2. The roots were mainly thin and wiry. Most of them were between 30 and 50 cm. deep. A few turned downwards and reached a maximum depth of 146 cm. There was no deep tap root. The relative concentration of roots between 30 and 50 cm. may be the combined result of the surface irrigations and the cover crop.

3. The ratio of branch weight to root weight was 1·99. The relatively high amount of branch to root on this sandy soil is probably largely due to the artificial irrigation, combined with the other climatic factors.

4. A hundred and seventy-five three-year-old apple trees of fourteen varieties, all on Canada Baldwin piece roots, were excavated at the Dominion Experimental Station, Summerland, British Columbia. Great variation was

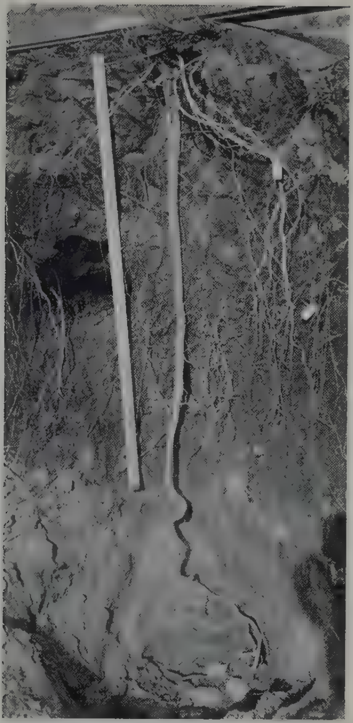


FIG. 1.
Soil profile in orchard at Summerland, B.C., showing underlying sand, and deep tap root of alfalfa (lucerne) cover crop. Length of measure = 1 metre.

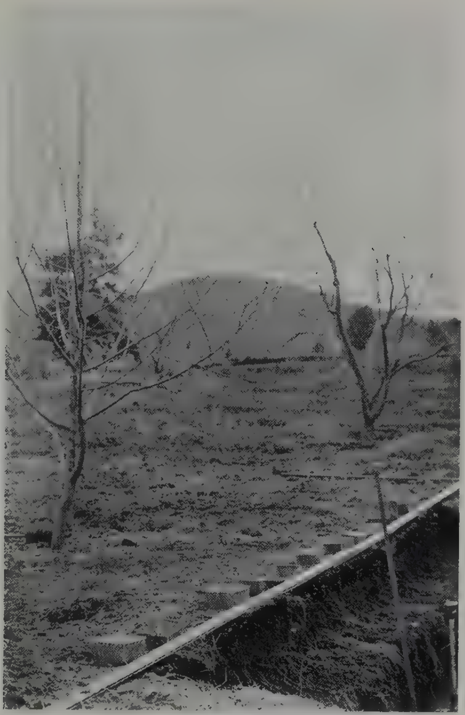


FIG. 2.
Eight-year-old McIntosh Red apple, showing excavation trench.

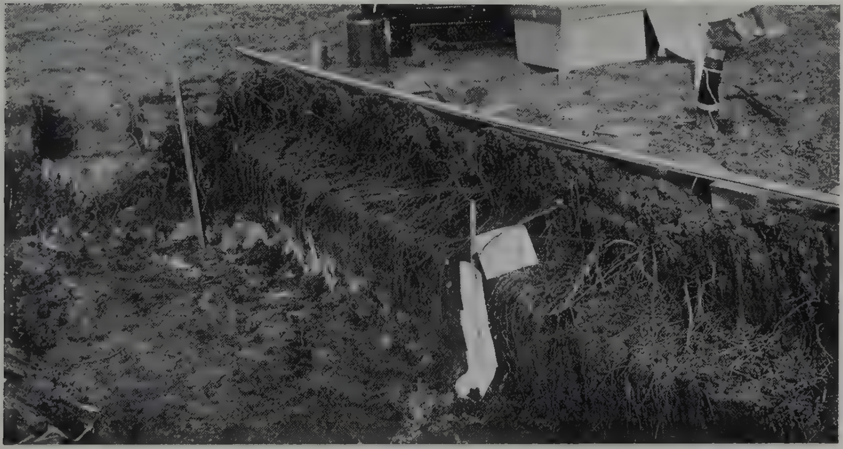
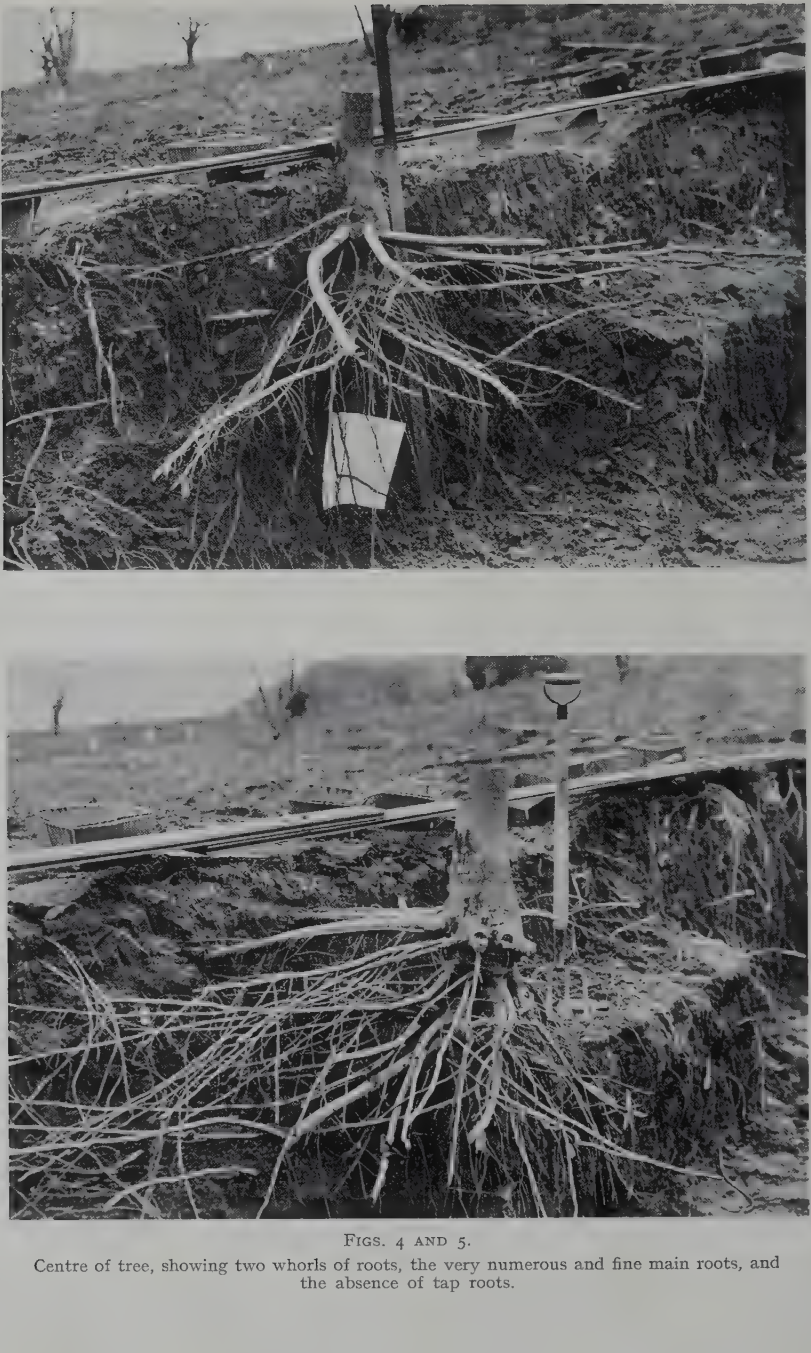


FIG. 3.
Excavation trench nearer the tree, showing fine roots and dyeing technique.



FIGS. 4 AND 5.

Centre of tree, showing two whorls of roots, the very numerous and fine main roots, and the absence of tap roots.

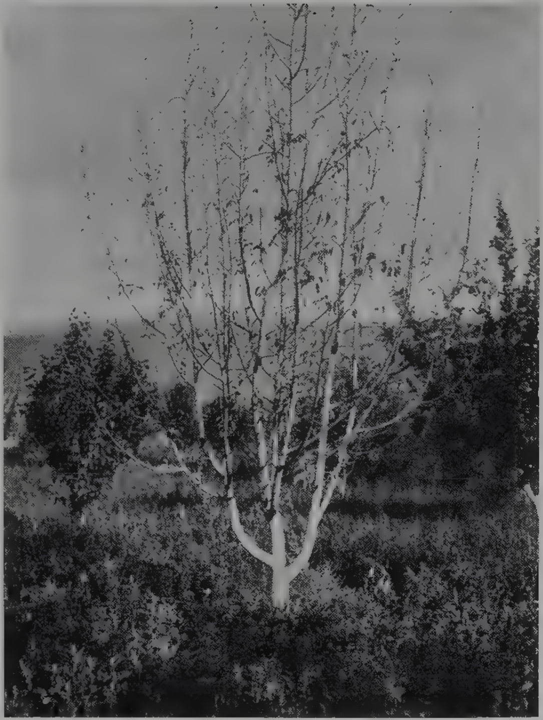


FIG. 6.

Winesap apple tree badly affected with " Collar Rot ".

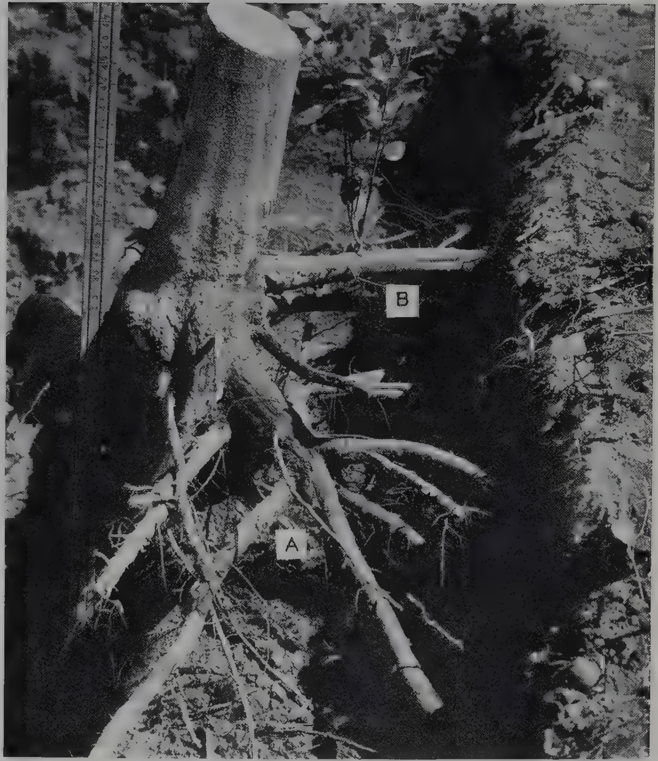


FIG. 7.

Centre of twelve-year-old Winesap with " Collar Rot ".

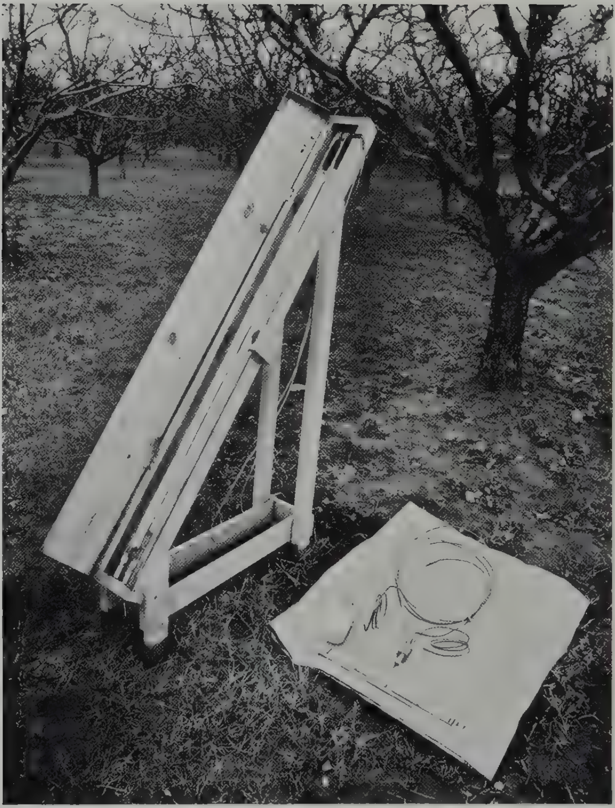


FIG. 8.

Soil moisture meter. The case contains two manometers connected to porous pots at different depths. Specimen porous pots and fittings are shown on the ground beside the instrument.

found between the root systems, even when grafted with the same variety. None of the varieties exercised a readily visible qualitative effect on the seedling roots.

5. Some comparable trees on Malling Clonal stocks had root systems very different from the seedling root systems.

6. In some varieties 100 per cent. of the trees on seedling roots had begun to form scion roots. The amount of scion root was very small at this age, however. Nine out of fourteen varieties showed some scion rooting.

7. Both roots and branches showed remarkable vigour under these irrigated conditions. Roots reached a length of 5 ft. and a depth of 3 ft. 6 in. in two years after planting.

8. A twelve-year-old Winesap apple tree suffering from "Collar Rot" was excavated. It was found that most of the main roots were dead at the crown. Some were dead throughout, and some alive below the crown.

9. A continuous-reading soil moisture meter for use in the field is described. Examples are given of its working. The capillary pull corresponding to optimum growth conditions is discussed.

REFERENCES.

(1) *Rogers, W. S., and Vyvyan, M. C.* Root Studies. I. The Root Systems of some ten-year-old apple trees on two different rootstocks and their relation to tree performance. East Malling Res. Sta. Ann. Rep. for 1926-7 (II Supplement) (1928), 31-43.

(2) ——— Root Studies V: Rootstock and soil effect on apple root systems. Journ. of Pom. & Hort. Sci., 1934, XII, 110-50.

(3) *Rogers, W. S.* Root Studies II: The root development of an apple tree in a wet clay soil. Journ. of Pom. & Hort. Sci., 1932, X, 219-27.

(4) ———. Root Studies IV: A method of observing root growth in the field; illustrated by observations in an irrigated apple orchard in British Columbia. East Malling Res. Sta. Ann. Rep. for 1933 (1934), 86-91.

(5) ———. A Soil Moisture Meter depending on the capillary pull of the soil, with illustrations of its use in fallow land, grass orchard and irrigated orchards. Journ. of Agric. Sci., 1935, XXV.

(6) *Veihmeyer, F. J., and Hendrickson, A. H.* Soil moisture conditions in relation to plant growth. Plant Physiology, 1927, II, 71-82.

(7) *Heck, A. F.* A soil hygrometer for irrigated cane lands of Hawaii. Journ. Amer. Soc. Agron., 1934, XXVI, 274-8.

LEAF RELATIONS OF FRUIT TREES

II. THE CENSUS METHOD FOR RECORDING SUMMER GROWTH, WITH SPECIAL REFERENCE TO THE APPLE

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A. INTRODUCTION.

This paper is a brief description of a rapid method for recording summer growth on trees.

The method consists in classifying the growths on the tree into categories on the basis of obvious morphological or physiological characters; each category is given a letter of the alphabet as a distinctive symbol. Starting at the apex of a tree, each growth in turn is examined and its category recorded; the result is a map of the tree and a census of the numbers of growths of each category. Provided appropriate characters have been chosen, differences due to response of trees to treatments are revealed by the census and can be proved statistically. The work is reasonably rapid; thirty or more four-year apple trees can be dealt with in a day by a pair of workers. In the case of larger trees, sample branches can be used.

The rather tedious task of going through the record sheets and adding up the numbers of growths of each category can be avoided by using the " categorimeter " or adding typewriter, which is described in another paper (19). This automatically adds up the numbers on each tree at the time the maps are made.

The choice of characters on which to base the categories must depend upon the morphological peculiarities of the type of tree used. As an example of general principles, the classification found convenient for the Apple is described in Section C ; the details would have to be modified for trees of different habit, such as plums or cherries.

A full description of the technique of recording will be given in Section D.

B. RECENT WORK ON SUMMER GROWTH.

No attempt will be made here to review the numerous papers published in recent years on summer growth. Reference will only be made to these papers where the methods used or the results obtained in them have a bearing on the choice of characters for the classification of growths, or the morphological analysis of the shoot system of the tree.

All summer work is subject to two difficulties not met with when measurements are made in winter; the rate of growth makes speed imperative, whilst the presence of leaves and fruit makes speed more difficult to attain.

Some workers have met these difficulties by confining observations to extension growth of shoots (e.g. 3, 8, 9, 20) chosen either for similarity in position or typical character (e.g. 6). Other workers have concentrated on following the history of a number of marked spurs (e.g. 14, 21, 25). Swarbrick (21) has used a mapping method when comparing the performance of spurs in successive seasons.

In a former paper of this series (24), where a complete morphological analysis of the tree was attempted, the difficulties were met by using only two trees and by waiting till rapid growth had ceased.

In the method described here, speed is attained by a reduction of actual measurements to a minimum and by the use of a simplified nomenclature. The categories are based on characters that can be recognized at a glance and are known by letters of the alphabet.

C. CLASSIFICATION OF APPLE GROWTHS.

The morphology and life cycle of the apple has been described by several authors (e.g. 4, 5, 7, 11, 18), but they do not all agree in their terminology; the terms used here will therefore be defined and illustrated.

The characters used in the classification and the symbols assigned to the categories are summarized in Table I.

TABLE I.

Categories of Apple Growths and their Distinctive Symbols.

	Bearing.	Non-bearing.			
		Growing.		Non-growing.	
		Short.	Long.	Short.	Long.
Primary	f, k	r	e	v	l
Secondary from Bearing	p	b	s	x	z
Secondary from Non-bearing Tertiary		a	t	g	h

OTHER USEFUL SYMBOLS.

q dormant bud. d dead growth. o broken off. – position of pruning cut. . .. ... etc., end of 1 year, 2 year, 3 year, etc., wood. , end of secondary (from bearing) wood. ( ) beginning and end of secondary (from non-bearing) wood.

The Characters.

(i) *Primary and Secondary.*

Primary growths are those developed from buds formed the previous year and dormant during the winter ; they are borne either terminally on last year's shoots or spurs or laterally just above the scars of last year's leaves, in whose axils they were formed.

Secondary growths are those that grow out from the axils of current year leaves borne on primary growths. Secondaries are further subdivided into those borne on growths that have blossomed (Bearing growths) and those borne on growths that have not blossomed (Non-bearing growths). The one arise in the normal course of the life cycle in all varieties, the other normally from strong leaders of some varieties such as Cox, and as a result of accidents or summer pruning in any variety.

Tertiary growths occasionally arise in the axils of leaves on the secondaries. To avoid increasing the number of categories and symbols, these can be conveniently classed with the secondaries from non-bearing growths.

This character of "order" or position is ideal for the purpose of classification ; morphologically the growths are easy to recognize, and physiologically they are probably of different value, as they grow out and form leaf surface at different times in the year.

(ii) *Bearing and Non-bearing.*

Bearing growths are those that produce at least one blossom or blossom bud during the season.

Non-bearing growths are those that produce none.

The presence of a blossom has important morphological consequences in the apple apart from its significance from the point of view of yield. The terminal growing point of the growth is used up and no further leaves and internodes can be formed. Increase in length can only occur through elongation of internodes already present. Secondary growths usually develop from the axils of some of the leaves.

Where no blossom is formed the terminal growing point is still present and can form an indefinite number of new leaves and internodes.

(iii) *Growing and Non-growing.*

Growing growths are those that have still an indefinite number of unflattened leaves at their tips.

Non-growing growths are those that, for the time being at any rate, have formed their last functional leaves.

This distinction is not as clear-cut as those above, but is very important, for the relative numbers of growing and non-growing growths form one of the best measures of the activity of a tree and its response to experimental conditions.

There is evidence too (3, 20), that it is the time when growth ceases, rather than the total amount of growth made, that determines the time and degree of fruit bud formation.

When a growth begins to develop, its leaves are at first rolled or folded upon one another, and it is impossible, by mere inspection, to say how many there will be (vide Fig. 5). As time goes on, each leaf in turn from the base upwards detaches itself from the bud and flattens out. It may remain partially rolled at margins for a while after it has become free, or it may be attached for a while by one margin after the rest of the blade has become flat. New leaves may form within the bud as those at the base flatten out, and this growing condition occasionally continues until the onset of winter kills the young leaves before they have flattened out. This is commonest in shoots that have broken into new activity in late summer after an early check. Usually, however, at some time in the season it becomes evident that no new leaves are being formed and those at the tip can be counted. Finally the last of these leaves flattens out and after a while ceases to grow.

The change from the growing to the non-growing condition is thus a gradual process. It may be said to start when the growing point lays down the leaf initial that will be the last functional leaf, and to end when this leaf has flattened out and ceased to grow. Neither the beginning nor the end can be readily recognized by inspection; hence some more clearly marked intermediate point must be taken as the dividing line between growing and non-growing. The time when the final number of leaves can be counted is one such point, the time when the last leaf has flattened out another. It does not matter which is taken, provided the same one is used consistently throughout the investigation.

When the last leaf flattens out, the tip of the growth is sometimes found to be capped by a conical bud clothed with scales (vide Fig. 6); sometimes the change is more gradual, the loose green bud becoming progressively attenuated until it is represented by a few green threads, the last of which may shrivel without flattening into leaves. Some judgment is necessary in these cases when deciding whether the growth is to be classed as growing or non-growing.

If only one census is to be taken, it is sometimes useful to distinguish three degrees of activity, growing, ceasing growth and inactive, the middle class including all those growths that are clearly at the point of ceasing growth, or, by the colour of the apical leaves, have recently been growing. These correspond more or less with the three classes recognized by Finch (6): growing, last internode formed, last leaf formed.

(iv) *Short and Long.*

Short growths are those less than 5 cm. in length, measured to the tip of the uppermost internode.

Long growths are those of greater length.

In the case of non-growing growths this seems a natural division, corresponding to the spurs and shoots of pomological nomenclature. In the case of growing growths it is arbitrary but convenient. A pencil with a notch cut 5 cm. from the base is a convenient measure for deciding borderline cases. The longer growths can be further subdivided, if desired; for example, the growing primaries can be called e1 when 5-10 cm., e2 when 10-15 cm., and so on. It is not advisable to make the units less than 5 cm., and longer units such as 5-15 cm., 15-25 cm. are more convenient. The larger the unit, the fewer the borderline cases that have to be carefully measured, and the greater the speed.

The Categories.

"f."

The primary bearing growths, developed in Spring from the "fruit" buds of pomology, are termed "f's". These are either "terminal", "spur" or "axillary" according to whether they have been developed from the terminal buds of last year's shoots or spurs, or formed from buds in the axils of last year's leaves. Terminal and spur "f's" are very alike and bear up to six or seven blossoms. A typical terminal "f" is shown in Fig. 1. Axillary "f's" are usually much weaker and have fewer blossoms, sometimes only one, e.g. Fig. 3.

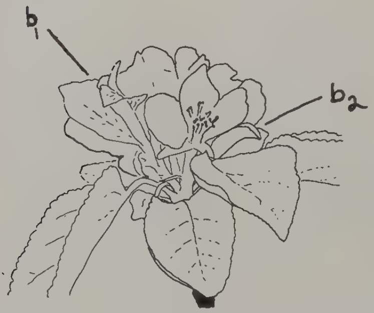

Fig. 1.
A terminal primary bearing growth, or "f", giving rise to two short growing non-bearing secondary growths, or "b's", which are already beginning to project beyond the petals of the blossom.

Axillary "f's" are occasionally large and indistinguishable, except in position, from those on spurs; some of these at any rate are really terminal on axillary growths that bore leaves the previous season, the "g's" of last year (10.11).

The internodes of an "f" frequently lengthen and thicken up to form a club-shaped structure called a "bourse" or a "cluster-base" (vide Figs. 1 and 2). One or more secondaries usually grow out from the axils of the leaves immediately below those subtending the lower blossoms. The form of the bourse (17) and the number of secondaries depends on the variety and on the position of the "f". Terminal and spur "f's" are usually easy to recognize even in autumn; axillary "f's" that have set no fruit may sometimes be mistaken for "v's", if no secondary has developed, and the "s" or "z" arising from one may be mistaken for an " e " or " l ", if the blossom scar has become obliterated by the thickening of the axis of the secondary.

" *k*."

In the middle of June 1935, possibly as a result of the destruction of normal blossoms by the severe frost in the middle of May, a large number of primary growths developed and opened blossoms at their tips. These bearing growths were clearly quite different from the normal " f's " developed in spring from fruit buds formed the previous season, neither can they have been a case of premature expansion of the normal fruit buds for the coming season, for these should not have been initiated until July or August.

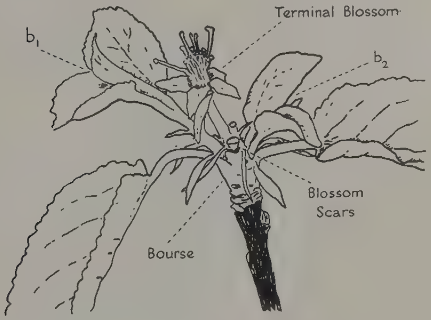

FIG. 2.
Same growth after removal of all the blossom except that formed from the terminal growing point, and all leaves except those subtending the two secondaries. *Notation :* fbb.

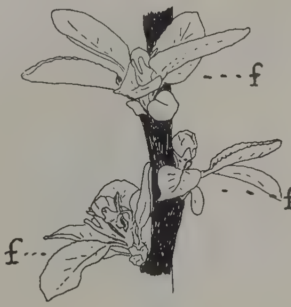

FIG. 3.
Three axillary primary bearing growths, photographed on the same day, on the same tree, as the terminal shown in Fig. 1. No secondaries showing yet.
Notation : f f f.

A typical example is shown in Fig. 4. They seemed to be something abnormal called forth by an abnormal season. They were particularly common on Cox and Laxton Superb. What appears to have been a similar abnormal blossoming on terminals after a late frost has been described by Vinson (23). Miedzyrzechi (16) has described a similar type of growth in the pear. These growths were given the symbol " k " in the censuses where they were found. In the censuses taken earlier in the season these growths appeared as " r's " or " e's ". The secondaries arising from " k's " were placed in categories " a ", " t ", " g " and " h ", as they resembled those from non-bearing primaries more than those from " f's ".

" *r*."

Every non-bearing primary growth starts as an " r " (Fig. 5), for until its leaves flatten out it is impossible to be sure how many there are. Some continue to produce new leaves within the bud as those on the outside flatten out. The outer leaves are separated by very short internodes and form a basal rosette, the later formed leaves may have longer internodes which may attain a considerable length before the leaf has flattened out. An internode does not seem to increase appreciably in length after the leaf above it has flattened out. These growths are still called " r's " until the length, measured to the tip of the uppermost visible internode, attains the length of 5 cm.; they then become "e's". The vast majority of growths on most varieties, including Cox, either cease growth long before they reach this length or else become much longer, and it is not often necessary to make an actual measurement except during the few weeks when the future " e's " are passing the 5 cm. mark.

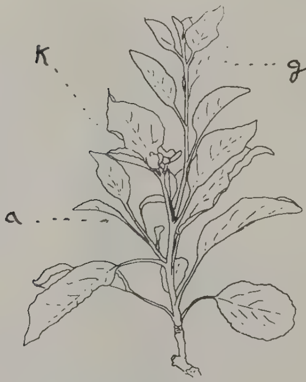

Fig. 4.
Example of a " k ", or late formed primary bearing growth. Two growing secondaries may be seen, one long " g ", and one short " a ".
Notation : kga.

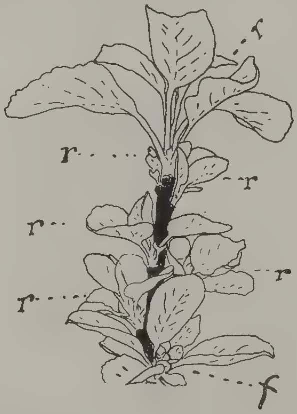

Fig. 5.
Terminal non-bearing primary, followed by five axillary non-bearing and one axillary bearing primary. All the non-bearing still growing and under 5 cm.
Notation : rrrrrrf.

Growths that have become " v's " may break into new activity and become " r's " once more (Fig. 6).

" *e.*"

This symbol denotes a non-bearing primary growth that is 5 cm. or more in length and has still an indefinite number of unflattened leaves at its tip.

" *v.*"

A primary non-bearing growth that has ceased to form new leaves before attaining a length of 5 cm. is denoted a " v ". More than one type of spur is included in this category ; some growths produce only a few leaves, or even only one, and show no trace of a central axis or terminal bud (vide Fig. 9) ; and when the leaves fall early in the season, as they frequently do, there is nothing to show that the bud has produced a growth. Others produce a rosette of larger leaves, often with a large central conical bud (Fig. 6). Others again, produce a considerable axis several centimetres long ; these are commonly called " dards ". The various types, however, tend to grade into one another and there is a limit to the number of categories that can conveniently be recognized during the period of rapid growth when speed in recording is essential. If desired, a more leisurely census can be taken in autumn, when growth is nearly over, and the various types of " v " denoted by distinctive symbols.

" l."

A non-bearing primary growth 5 cm. or more in length is called an " l " when it has formed its last leaf. Growths of this type may become active again later in the season and become " e's ". The position of the " check " is usually marked by a ring of bud scale scars where the temporary resting bud was formed. Sometimes there may be more than one check in a season. As mentioned above under "k", blossom may be produced when an "l" breaks into new growth in late autumn.

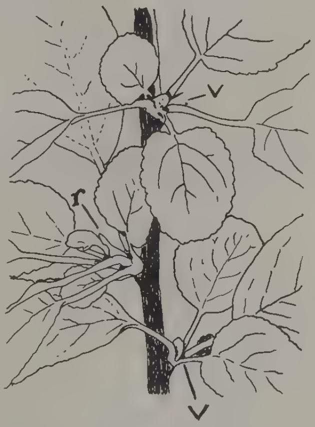

Fig. 6.
Three short non-bearing primary growths. Upper and lowest non-growing with conical terminal resting buds. Middle growth breaking into renewed activity after period of rest.
Notation : vrv.

" p."

This denotes the secondary blossom bearing growth that sometimes grows out from the bourse of a primary bearing growth. These growths vary in size from a short axis little thicker than a normal blossom stalk and bearing one leaf and one blossom, to a structure almost as important as the primary growth itself, and frequently carrying tertiary growths. Several authors have described these growths (4, 22, 23). The time of differentiation of the blossoms on these growths is not known for certain (23). Similar growths frequently arise on pears (16).

If there is only one blossom this frequently falls off as a small aborted bud. In general the blossom usually opens later than those on the primaries.

Sometimes a growth of this type gets entered as a " b " at an early census and is only recognized as a " p " at a later one.

In 1935 numerous large secondary bearing growths developed, especially on Cox. Although classed as " p's " these were probably abnormalities like the " k's " produced by the late frost. Similar abnormalities following a late frost have been described in America (1, 22, 23). An example is shown in Fig. 7.

" b."

All non-bearing secondary growths developing from primary bearing growths start as " b's ", that is with an indefinite number of unflattened leaves.

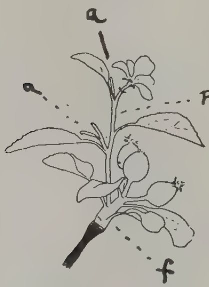

FIG. 7.

Example of a "p", or secondary bearing growth. This has developed from the axil of a leaf on the " bourse " of a primary bearing growth "f", which is carrying three fruits. Two tertiary " a's " are growing out from the " p". *Notation:* fpaa. *Note:* " p's " are usually much smaller and less conspicuous than this one.

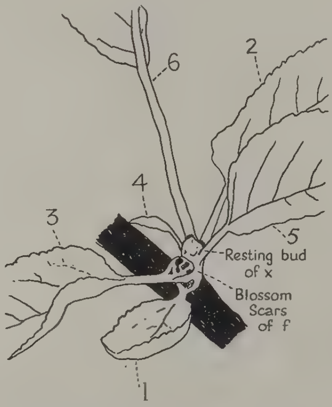

FIG. 8.

An axillary " f ", or primary bearing growth, terminating in blossom scars, and carrying three leaves (1, 2 and 3). An " x ", or non-bearing non-growing short secondary, is present in the axil of leaf 2, and carries three leaves (4, 5 and 6) and a terminal resting bud.

The number borne by a bourse depends on the variety. The position of the bourse on the tree may affect their number, their time of development, and their ultimate length. The " f's " shown in Figs. 1 and 3 were photographed on the same day; the "b's" from the terminal (Fig. 1) were already projecting beyond the blossoms, whilst those on the axillaries (Fig. 3) were not yet visible even as buds.

"s."

When a " b " passes the 5 cm. mark it becomes an " s ". The change usually takes place rather later in the season than the change of a primary from an "r" to an "e". When a strong leader on a young tree has been pruned back to a fruit bud, as frequently happens on a precocious stock like No. IX, two or three strong secondary leaders may grow out from the apical surviving bourse, giving the tree the bifurcating habit so characteristic of trees on No. IX (vide 12). In some varieties the basal leaves are crowded into a rosette; in others such as Cox, the internodes above the basal leaf may be much longer than in a primary growth.

"x."

When a "b" ceases growth before reaching 5 cm. it becomes an "x". These secondary spurs vary in strength, like the primaries, from single leaves to strong "dards" several centimetres long. A typical "x" is shown in Fig. 8.

"z."

When an "s" ultimately ceases growth and forms no more leaves it is termed a "z". A "z" is usually shorter than an "l" borne in a comparable position; its length is said to be largely influenced by the set of fruit.

"a", "t", "g" and "h".

These are respectively the growing short ("a") and long ("t"), and non-growing short ("g") and long ("h") secondaries from non-bearing primary growths. They may vary from the solitary leaf, often found in an axil on a primary even on mature trees, to long vigorous shoots. Physiologically these growths are probably distinct in origin and in value to the tree from the secondaries arising from bearing growths. Those arise from growths whose growing points have been used up in the formation of blossom, these through superabundance of vigour from growths that are often themselves in vigorous growth. They do not usually develop moreover till much later in the season.

These same symbols are also used to denote the tertiary growths (vide Fig. 7).

D. DETAILS OF METHOD FOR MAPPING TREES.

The census is conveniently taken by two workers, one to observe, the other to record. Starting at the top of the tree or branch, the observer examines each growth in turn and calls out its category; the recorder notes this down. By taking the growths thus in their natural sequence, the danger of missing one or recording one twice is lessened. There is the further great advantage that the records provide a complete plan of the tree, and any growth shown on the records can be subsequently identified on the tree if further observations are required.

It is a great advantage for later observations if the records are subdivided to correspond to the major and minor subdivisions of the branch system by the use of various types of stops and brackets; this involves very little extra work at the time and provides much additional information. It is particularly useful if several censuses are to be taken in the same season, for the successive records can be entered one below the other on the same record sheets and the history of each individual growth can be followed through the season and correlated with its performance next spring. The notation found convenient for the apple is best illustrated by following the history of a tree or branch system over a number of years.

Non-bearing Primaries.

The simplest example, where all the growths are non-bearing primaries, will be taken first.

First or Maiden Season.

The tree during its first year of growth consists of the single non-bearing primary, developing from the scion bud. This starts as an " r ", develops into an " e " and finishes the season as an " l ". Provided no secondaries grow out, these three symbols written one below the other, each opposite its appropriate date, will provide a complete map of the tree and its history during the season.

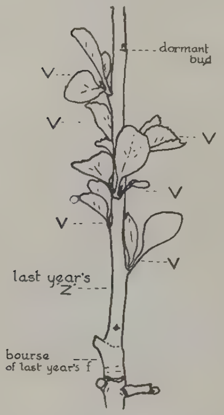

FIG. 9.

Six non-growing non-bearing short primary growths, borne on last year's secondary shoot from last year's terminal bearing growth. *Notation :* (including the dormant bud visible above the growths) "qvvvvvv ,.".

Second Season.

At the beginning of the second season the tree consists of a single one-year-old stem, last season's " l ", bearing one terminal and a number of lateral or axillary dormant buds, developed in the axils of last year's leaves. Most of these axillaries will be well spaced and easy to count, but those at the base will be crowded and indefinite in number. These dormant buds are the potential growing points for the coming season, and their number is a measure of the initial size of the tree.

The mapping of such a tree after bud-break is simple. Starting at the terminal, the category of each successive growth is written down in turn till the basal growth is reached. The resulting map might read rrrrrrrrrqqrr. Dormant buds are indicated by the symbol " q " which reserves a place on the plan for a growth that may subsequently develop. Incidentally these " q's " form useful reference points on the tree. These dormant buds need only be indicated if they occur in an intermediate position between buds that have broken into growth. Dormant buds below the lowest growth are not recorded on the map, but a space is left between the final growth and the dot " . " which denotes the position of the bud scales at the base of the one-year wood.

As the season advances, some of the " r's " will grow out into " e's " and finish "l's"; others will remain short and become " v's ". The complete map from three successive censuses, taken in Spring, Summer and Autumn, might read :—

rrrrrrrrrrqqrr .
eeeelvvvvvqrev .
1111lvvvvvqvlv .

Third Season.

At the beginning of the third season the tree is represented by a branch system consisting of a central trunk—the two-year-old stem—and a number of one-year-old shoots and spurs representing the " l's " and " v's " of last year. Each has its terminal and axillary dormant buds. When taking a census on such a tree, the growths on the uppermost one-year-old shoot are recorded first, and followed by a dot. Then those on the next one-year-old stem, followed by another dot, and so on until the base of the two-year-old wood is reached ; this is indicated by two dots thus " . . ". Such a record might read :—

rrrr .rrr .rrr .rrr .rr .r .r .r .r .r .q .r .rr .r . .

Later Years.

The same procedure is followed in older trees. The growths are recorded in succession from the tip of the uppermost shoot to the base of the lowest, and the position of bud scale scars separating years of growth indicated by dots—" . " for the base of one year wood, " . . " for the base of two-year wood, " . . . " for the base of three-year wood, and so on.

Symbol for Pruning Cuts.

Growths often behave differently on pruned and unpruned shoots; the presence of a pruning cut should therefore be indicated. This can be conveniently done with a hyphen. Thus -rrrrr would mean a pruned one-year-old shoot whose five uppermost axillary buds had grown out, whilst rrrrr . would mean an unpruned one-year shoot whose terminal and four uppermost axillary buds had grown out. Older pruning cuts can logically be indicated in the same way, thus -.-rrr . might describe the growths on a pruned one-year-old shoot rising from the apical surviving bud on a pruned two-year-old stem. It is unnecessary, however, to indicate pruning cuts more than a few years old.

Secondaries on Bearing Growths.

Current Year.

When recording an " f ", or primary bearing growth, on the plan, it is advisable to leave a gap for secondaries that may develop from it. Thus if the third growth is an " f " and the first, second, fourth and fifth are " r's ", the plan would read rrf rr . Later it might become eefbb vv ., perhaps finishing as llfzx vv. The size of the gap will depend on the maximum number of secondaries to be expected, and that will depend on the variety.

Past Years.

It is useful to record on the plan if last year's growth was a secondary " z " or "x"; this can be readily done by using the comma " , " to mark the end of a secondary borne on a bearing growth. Thus the llfzx vv . of one season might become rrr .rrr .rr ,r ,.r .r . . the next Spring ; the " rr ," indicating two growths borne on the " z ", and the " r ," one growth borne on the " x ". This notation is particularly valuable when recording old spurs, as it reveals any biennial or regular bearing habit. For example the spur shown in Figs. 10 and 11 would be recorded thus : " v , . . , . . , ." and the past history of the spur can be reconstructed as follows :

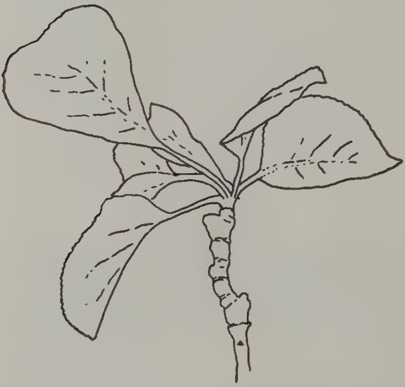

FIG. 10.

A " v ", or short non-growing non-bearing primary growth carried terminally on last year's short secondary growth. The scars on the old wood (shown on a larger scale in Fig. 11) prove that the spur has been bearing blossom in alternate years. The photograph was taken in 1934, when the spur was in an off year. Blossoms were borne in 1933, 1931 and 1929.

Notation : v , . . , . . , .

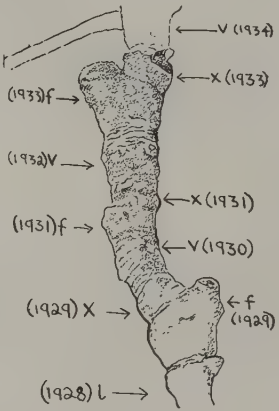

FIG. 11.

Old wood of spur shown in Fig. 10, on a larger scale to show past history of the spur. Notation in successive years would have been as follows : 1928 " l ". 1929 " fx ". 1930 " v , . ". 1931 " fx . , . ". 1932 " v , . . , . ". 1933 " fx . , . . , . ". 1934 " v , . . , . . , . ".

1929	1930	1931	1932	1933	1934
fx	v , .	fx . , .	v , . . , .	fx . , . . , .	v , . . , . . , .

Thus the spur had shown a consistent biennial habit, producing blossoms in alternate years. If it had been persistently vegetative, producing a " v " each year, the record would have read: " v . . . . . ". If it had been persistently bearing, producing " fx's " each year, it would have read " fx , . , . , . , . , . ".

Secondaries on Non-bearing Growths.

The mapping of these presents difficulties, for more than a dozen may rise from a single primary, and it is not convenient to leave a gap in the plan for them as is done for the secondaries from bearing primaries. In practice, up till now, they have not been recorded, but it is becoming evident that they will have to be. The number borne on a primary shoot depends on its vigour; it is highest on strong leaders and on vigorous stocks, and it may prove a useful index of response to manurial treatments.

The method now adopted is as follows: Only a small proportion of the primary growths produce secondaries; these would be underlined on the plan. Thus, "eeeelv" would mean that the first two " e's " had produced secondaries. A separate census will then be made of these underlined growths alone, giving each ample room on the secondary map. For example, the new map for these two " e's " might read:

e aaaaa/aaa e aa/qaqa

The symbol / representing the position of a spot of paint or a thread tied round the stem to serve as a reference point.

Growths borne the next year on a secondary of this type are enclosed in brackets to indicate that they are on a side shoot. For example the record " rrrrrrr(rrrr)rr ." would denote a one-year-old primary stem carrying seven " r's " above a secondary, two below it and four on the secondary itself.

Old Trees.

When mapping old trees it is often impossible to know which is the upper and which the lower limb at an important fork. If there has been much cutting back into old wood in past years, buds may have been forced into growth after several years of dormancy, introducing further confusion. A judicious use of numbered labels is a great help on these trees. The labels should be placed near the centre of long runs of similar growths, rather than at actual forks, as they then serve as additional reference points as well as indicating which limb was dealt with first.

E. EXAMPLES OF RESULTS FROM CENSUSES.

Censuses were taken at the beginning of June in 1933 and 1934 on sixty-four trees of Cox's Orange Pippin on the New Manurial Trial at East Malling (13), and further censuses are being taken in 1935. The full results are being analysed statistically and will be published in a later paper, but two examples of results will be given here to show the type of facts that the census method will reveal.

The trees used for the censuses were on four stocks (Nos. I, V, IX and XII), under four different manurial treatments (O, N, K and NK) ; four replicates of each of the sixteen stock-treatment combinations were taken, making up a total of sixty-four trees.

In 1933 the whole tree was taken as the unit, every growth being recorded ; in 1934 one branch, roughly one-third of the tree, was used as the unit.

Example i.

The total number of primary and secondary, bearing and non-bearing growths borne on the sixteen trees of each stock are summarized in Table II. The secondaries are those on the bearing primaries, none had yet grown out from the non-bearing growths.

TABLE II.

Number of Bearing and Non-bearing Primary and Secondary Growths on Sixteen Trees of Cox on each of four different Rootstocks.

Year		1933		1934	
Age		4 years.		5 years.	
Unit		Whole Tree.		One Branch.	
		1 ry.	2ndy.	1ry.	2ndy.
I	B.	156	4	642	7
	NB.	1,476	234	1,217	928
V	B.	217	10	519	6
	NB.	1,317	330	1,308	830
IX	B.	497	25	663	29
	NB.	792	745	651	770
XII	B.	2	—	13	—
	NB.	2,089	3	2,903	24

The main points of interest revealed by this table are :

(1) One branch in 1934, on an average, carried more growths than the whole tree in 1933.

(2) The relative numbers of bearing and non-bearing growths differed widely on different stocks, No. IX having the highest and No. XII the lowest relative numbers of bearing, as might be expected.

(3) The relative numbers of primary and secondary growths were widely different on different stocks, this follows naturally from the differences in the relative numbers of bearing and non-bearing primaries.

Example ii.

In 1933 a second census was taken on the same trees at the end of July; the growths can therefore be divided that year into those that had ceased growing before June, those that ceased growth between the beginning of June and the end of July, and those that did not cease growing till after the beginning of August. This has been done in Table III.

TABLE III.

Number of Growths that ceased growing before the beginning of June, during June and July, and after end of July, 1933.

Stock.	Ceased Growing.	1ry.	2ndy.	Total.	Per cent.
I	Before June	834	140	974	57
	During June and July ..	315	47	362	21
	After July	327	47	374	22
V	Before June	729	202	931	56
	During June and July ..	258	68	326	20
	After July	330	60	390	24
IX	Before June	344	175	519	34
	During June and July ..	349	381	730	48
	After July	99	189	288	19
XII	Before June	1,068	3	1,071	51
	During June and July ..	505	—	505	24
	After July	516	—	516	25

The chief point of interest is the difference in behaviour of trees on No. IX from those on other stocks. Most of the growths on No. IX became inactive during June or July, the proportion ceasing to grow before the beginning of June being much smaller than on other stocks. The proportion that continued to grow after the end of July was also slightly less, which is in accordance with the observations made by Swarbrick at Long Ashton (20).

F. SUMMARY.

1. An easy method for rapid recording of summer growth is described.

2. Growths are classified into categories on the basis of easily recognized characters such as order (primary or secondary), performance (bearing or non-bearing), activity (growing or non-growing), and size (short or long).

3. Each category is given a letter of the alphabet as a distinctive symbol.

4. Each growth in turn from the apex to the base of the tree or sample branch is examined and its category recorded; the result is a census of growths of each category and a map of the tree.

5. The map is subdivided by full-stops, commas and brackets into sections corresponding to the major and minor subdivisions of the branch system.

6. Successive censuses can be recorded one below the other on the same sheet, and the history of individual growths thus followed through the season and correlated with their performance next season, and with their position on the tree.

LITERATURE CITED.

(1) *Auchter, E. C., and Knapp, H. B.* Orchard and Small Fruit Culture. New York. 1929.

(2) *Auchter, E. C., and Schrader, A. L.* Fruit spur growth and fruit bud formation. Proc. Amer. Soc. Hort. Sci., 1923, 127.

(3) *Barnard, C., and Read, F. M.* Studies of growth and fruit bud formation. I: A year's observations on Victorian Apples. Journ. Dept. Agr. Victoria (Australia), 1932, 349.

(4) *Bijhouver, J.* De Periodiciteit van de Knopontwikkeling bij den Appel. Meded. v. d. Landbouwhoogeschool, xxvii, 1923.

(5) *Boyes, D.* Notes on the character of apple tree shoots. Journ. Pom. & Hort. Sci., 1922, 3, 36.

(6) *Finch, A. H.* Physiology of apple varieties. Pl. Physiol., 1935, 10, 49.

(7) *Gardner, V. R., Bradford, F. C., and Hooker, H. D.* Orcharding. New York, 1927.

(8) *Gourley, J. H.* Studies in fruit bud formation. New Hamp. Agr. Exp. Sta. Tech. Bul. 9, 1915.

(9) ———. Some observations on the growth of apple trees. New Hamp. Agr. Exp. Sta. Tech. Bul. 12, 1917.

(10) ———. Fruit bud formation—a criticism. Bull. Torr. Bot. Club, 1917, 44, 455.

(11) ———. Textbook of Pomology. New York, 1922.

(12) *Hatton, R. G.* The relationship between scion and rootstock with special reference to the tree fruits. Journ. Roy. Hort. Soc., 55, 1930, 169.

(13) *Hoblyn, T. N.* Field experiments in horticulture. Imperial Bur. Fruit Prod. Tech. Comm. No. 2, 1931.

(14) *Hooker, H. D.* Annual and biennial bearing in York Imperial apples. Mo. Agr. Exp. Sta. Res. Bul. 75, 1925.

(15) *Maney, T. J., and Plagge, H. H.* Fruit bud production in the apple. Proc. Amer. Hort. Sci., 1920, 250.

(16) *Miedzyrzechi, C.* "Seconde floraison" chez le poirer. Journ. Soc. Nat. Hort. France, 1932, 5, 394.

(17) *Pearl, R. T.* The inflorescences of apple trees. II: An historical review together with further varietal descriptions. Journ. S.E. Agr. Coll., Wye, Kent, 1933, 9.

(18) *Roberts, R. H.* Off-year apple bearing. Wis. Agr. Exp. Sta. Bull. 317, 1920.

(19) *Rogers, W. S., and Vyvyan, M. C.* A device for the serial recording and automatic counting in categories. Journ. Pom. & Hort. Sci., 1935, 13.

(20) *Swarbrick, T.* The seasonal elongation of apple varieties on some vegetative root stocks and its possible relation to fruit bud formation. Journ. Pom. & Hort. Sci., 1928, 7, 100.

(21) ———. Biennial bearing of apples. Ann. Rep. Agr. & Hort. Res. Sta., Long Ashton, 1934, 37.

(22) *Vinson, C. G.* Secondary flowering of the apple. Proc. Amer. Soc. Hort. Sci., 1922, 173.

(23) ———. Secondary flowering of the apple. Proc. Amer. Soc. Hort. Sci., 1923, 37.

(24) *Vyvyan, M. C., and Evans, H. H.* The leaf relations of fruit trees. I. A morphological analysis of the distribution of leaf surface on two nine-year-old apple trees (*Laxton Superb*). Journ. Pom. & Hort. Sci., 1932, 10, 228.

(25) *Wiggans, C. C.* Some factors favouring or opposing fruitfulness in apples. Mo. Agr. Exp. Sta. Res. Bul. 32, 1918.

A DEVICE FOR SERIAL RECORDING AND AUTOMATIC COUNTING IN CATEGORIES

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THE " growth census " recording of tree shoots in various categories (1) produced the need for an apparatus for the rapid summarizing of the records. At first this was accomplished by having a number of counters (with zero resetting mechanisms) mounted on a board, each counter corresponding to one category. After obtaining the field records, the summarizer simply ran his finger down the record sheet, and pressed the appropriate counter for each observation with one of the fingers of his free hand, so getting the number of cases in each category. Even this technique proved quite laborious, and in order to avoid it, the following apparatus was made to count automatically the cases in each category, at the same time as they were recorded in their correct serial order.

This recording apparatus consists of a portable typewriter with reset counters connected to certain keys, so that the number of times the appropriate keys are pressed is counted while the record is typed on the paper. The counters are mounted on a wooden framework above the typewriter, so that they do not impede the operator's view of the paper, as shown in Fig. 1.

We are provisionally calling the apparatus a Categorimeter.

CONSTRUCTION.

The construction of the apparatus is clearly shown in Fig. 1. The following technical details contribute to its successful working. The typewriter is an Underwood Standard Portable. The keys of this machine are particularly convenient for the purpose. The two uprights fastened to the baseboard by triangular brackets of sheet steel are of straight grained deal $1\frac{7}{8}$ in. by $\frac{7}{8}$ in. by $19\frac{1}{2}$ in. The board to which the counters are screwed is $5\frac{1}{2}$ in. wide and $\frac{7}{8}$ in. thick.

The counters—of which fifteen can be accommodated on a framework of the size shown—are Findlay* 4 figure reset counters, with operating lever on the left side.

In order to get the correct stroke the operating arms of the counters are drilled with a $\frac{1}{16}$ in. hole centred $\frac{9}{16}$ in. from the shaft axis, and the arms are set at 50° from the horizontal.

The counters are screwed to the baseboard in sloping lines so that the operating wires hang clear of each other.

The wire making the connection between the keys and the counters must be stiff but not too thick. Steel wire No. 21 S.W.G. (·032 in. diam.) is suitable.

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* Messrs. Findlay & Co., Broadway Chambers, Hammersmith, London, W.6.

In order to get each length of wire straight, it was heated red hot by running a bunsen flame along it while it was pulled tight. The resulting degree of hardness proved satisfactory, and it was found possible to get an accurate length adjustment by careful bending at the ends.

The bottom end is bent sharply, making a hook $\frac{1}{16}$ in. wide, with a free end $2\frac{1}{4}$ in. long which projects upwards and prevents the wire from slipping forwards. This hook slips under the key bar, whose shape holds it in place. The top end makes a rather wider loop, which allows for adjustment. There should be about $\frac{1}{32}$ in. play in the wire when the key is not being pressed. This avoids errors when the machine is operated rapidly.

The extra load on the typewriter key caused by the counter is not great, but if desired a rather lighter spring can be substituted for the standard counter spring. Each counter is marked with the letter to which it is connected. The counters can be connected to practically any series of keys that are desired. They can also be changed from key to key, but in this case the wires will need readjusting. Unless screw adjustments are fitted to the wires it is simpler to use the symbols that are already connected to counters than to change the counters to other letters. Any number of counters can be fitted, but for more than fifteen a wider board is required.

Cost.

The Underwood Portable Typewriter costs £13 13s., and the counters cost 14s. 6d. each. The apparatus is therefore quite expensive to instal, but the saving in labour of summarizing is very great. In fact the apparatus makes possible work that could not otherwise be carried out. Complete instruments can be obtained from the Typewriter Exchange, Maidstone, Kent.

Operation.

The apparatus is light enough to be carried into the field, and can conveniently be used on a small table or an apple box. After the plot number and appropriate details are typed on the record sheet, all the counters are set to zero. In the case of the "growth census" records the observer calls out the letters corresponding to the growth category of each shoot, and the recorder types them. At the end of each branch or sample the totals on each counter are copied down, and the counters are reset to zero. In this case, where a series of records are made on the same branches, it is convenient to space the records several lines apart at the first recording, made with the black typing ribbon, and then to make the next record on the same sheets, using the red typing ribbon. The record for each shoot thus comes below the previous record for the same shoot, and it can be seen at a glance what changes have occurred, while the records made at different dates are readily distinguished.

Other records for which the apparatus is useful are health and disease surveys of plots of strawberries and similar plants, and fruit grading records, especially where various classes of damage are being recorded.

SUMMARY.

A recording apparatus consisting of a portable typewriter with reset counters attached to certain keys is described. This apparatus records observations represented by various letters in serial order and automatically counts the number of cases in different categories. In the case of growth census records or plant-to-plant surveys of plots a series of records of the same plants can be made on one sheet, showing the progress of each plant. The apparatus can be used indoors or in the field.

REFERENCES.

(1) *Vyvyan, M. C.* Leaf relations of fruit trees. II: The census method for recording summer growth, with special reference to the apple.

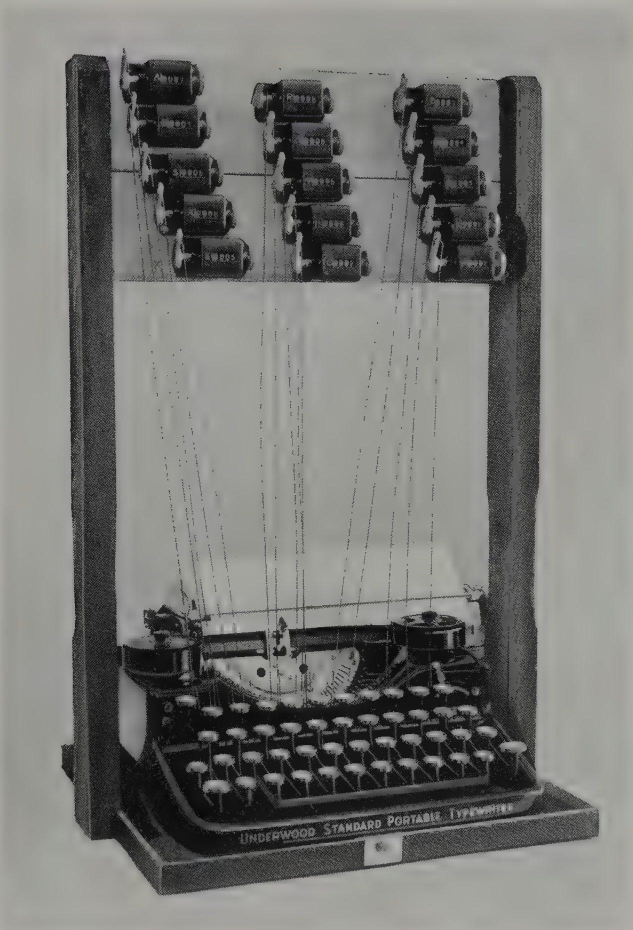

FIG. 1.
A "Categorimeter" or typewriter with counters for serial recording and counting objects in categories.

SOME ASPECTS OF THE ERROR OF ESTIMATES OF WASTAGE IN STORED FRUIT

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Fruit in storage always shows wastage, that is, one by one the fruits become diseased and unfit for consumption. Heavy losses are incurred annually, and many experiments have been made to discover and eliminate the cause of this wastage. In plan, these experiments vary somewhat in details, but all of them are fundamentally of the same type. In the simplest case a sample of fruit is treated in some way and is then stored for a given period, after which the wastage which has developed is compared with that developed in an untreated sample which serves as a control. Differences, if any, are then tested for significance by one of the usual statistical methods.

Experience with experiments of this type has shown that the standard error of the estimate of wastage in a sample tends to be high, and that, in consequence, comparisons of samples are usually trustworthy only when large amounts of fruit are used. This is both expensive and tedious. A study was therefore made of the factors which influence the magnitude of the error of estimates of wastage, and it is intended here to deal with some of them which are, perhaps, not immediately evident from the general literature of the planning of experiments.

The considerations to be discussed are general and apply to some extent to all types of wastage experiment. They are, however, intended primarily for experiments in connection with export fruit, which is packed in commercial boxes and stored for a period which is no longer than the time taken in commercial practice to bring the fruit from the grower to the consumer. Under these conditions it commonly happens that the time is insufficient for the development of large amounts of wastage ; from the experimental point of view this introduces a special set of problems.

It must be stressed that no attention is here given to that type of experiment in which fruit is stored for an unlimited period and wastage allowed to develop to proportions approaching 100 per cent.

General Considerations.

To estimate the error of an estimate of wastage it is necessary that observations should be replicated. For simplicity of argument it will be assumed that this is done by including several boxes of fruit in each sample, the boxes being considered as replicates. The total number of wasty fruits in the sample divided by the number of boxes estimates wastage as the mean number of wasty fruits per box. This estimate will be referred to as $\bar{x}$. The number of wasty fruits in each box is recorded separately, and from the scattering of these values about the mean, the standard deviation, s, is estimated by the usual formula. The standard error of the estimate of the mean is then estimated in the usual way by dividing the standard deviation by the square root of the number of boxes in the sample.

It is more useful to estimate the standard deviation, or the standard error, as a percentage of the mean than as an absolute quantity. The standard deviation as a percentage of the mean, i.e., $\frac{100\ s}{\bar{x}}$, will be referred to as the coefficient of variability, under which name it has been discussed by Hoblyn (2). In this form it serves as a measure of experimental efficiency, in the sense that it indicates the number of boxes which would be required to reduce the percentage standard error to such a value that an expected given percentage difference between samples could be demonstrated to be significant by one of the usual tests.

The magnitude of the coefficient of variability may be reduced in several ways ; fruit of more uniform quality may be used, the sample may be handled and stored in a more uniform manner, and so on. Methods of this type have their parallels in almost every horticultural or agricultural experiment. They have often been discussed and there is no need for elaboration here. In one respect, however, wastage experiments are rather unusual. In most horticultural and agricultural experiments the coefficient of variability is a constant for, and descriptive of, the particular set of experimental conditions used. In wastage experiments, however, its magnitude depends not only on the uniformity of the source and treatment of the fruit or on other experimental conditions, but also on the amount of wastage which is present. The coefficient is not a constant but, in a single experiment, varies progressively with the increasing wastage which develops during storage. With it varies the efficiency of the experiment, and differences between samples which cannot be detected at one stage may become significant later on.

This peculiarity of wastage experiments is, in statistical language, due to the fact that the distribution of wasty fruits from box to box is discontinuous.* Some of the consequences of this type of distribution do not appear to have been fully considered, and certain practical applications to wastage experiments require discussion.

The Effect of Increasing Wastage on the Coefficient of Variability of Homogeneous Fruit.

The simplest case for discussion is that of homogeneous fruit. This may be regarded as fruit taken from an absolutely uniform source and treated in an absolutely uniform manner. Variations in wastage from box to box would then be due only to the random scattering of wasty fruits throughout the sample. The complete uniformity required for absolute homogeneity is, of course, never attained in practice, but the results obtained on a postulate of homogeneity are nevertheless of considerable practical importance.

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* If two boxes differ at all, they must differ by at least one wasty fruit, infinitesimal differences being impossible. The distribution is therefore said to be discontinuous. The particular distribution concerned belongs, in the case of perfectly homogeneous fruit, to the binomial series. Details are given in R. A. Fisher's *Statistical Methods for Research Workers*, Oliver & Boyd, 1930.

In the case of homogeneous fruit the coefficient of variability may be estimated by direct calculation. The standard deviation, s, is estimated as $\pm \sqrt{\bar{x}q}$, where $\bar{x}$, as before, is the mean number of wasty fruits per box and q is the proportion of sound fruit. The coefficient of variability, $\frac{100\ s,}{\bar{x}}$ is therefore estimated as $\pm\ 100 \sqrt{\frac{q}{\bar{x}}}.$ Numerical values of this term, with the sign omitted, are plotted against $\bar{x}$ in the curve for homogeneous fruit in Fig. 1, the values being calculated on the assumption that the boxes contained a total of 150 (wasty and sound) fruits.

The curve shows that when the amount of wastage is very low, the value of the coefficient of variability is very high. Unless a large number of boxes of fruit were used in each sample, the percentage standard error would therefore also be high and, in consequence, a comparison of samples at this stage would be difficult. As wastage is allowed to develop, the value of the coefficient decreases continuously, at first rapidly but later more slowly. A comparison of samples would be facilitated correspondingly.

THE EFFECT OF INCREASING WASTAGE ON THE COEFFICIENT OF VARIABILITY OF ORDINARY (HETEROGENEOUS) FRUIT.

Data are also given in Fig. 1 for some ordinary, heterogeneous fruit. They were derived from a large experiment by Davies and Putterill (1), who kindly placed their figures at my disposal. The records are for green mould (*Penicillium digitatum* Sacc.) infections of Navel oranges picked from a single estate in 1931, and each point recorded in Fig. 1 is based on the information from about 180 boxes.

Fig. 1 shows that when the amount of wastage is very low the curves for homogeneous and heterogeneous fruit are concurrent, the two types of fruit being almost identical at this stage. With increasing wastage the curves diverge. The curve for the heterogeneous fruit falls less rapidly ; and after the value of the coefficient of variability has fallen a little below 60, the curve becomes almost flat. At this stage changes with increasing wastage are so small as to be negligible for practical purposes.

It is not intended to deal with the theoretical aspects of the curve, but it may be stated that an examination of the problem has shown that the type of curve given for the particular example of heterogeneous fruit in Fig. 1 is quite general. Curves for any other heterogeneous fruit will be of the same form and will differ only in numerical detail. All fruit, however heterogeneous it may be in origin and treatment, will always behave as if it were homogeneous when the amount of wastage is very low. Thus, for example, it can be shown that a test for heterogeneity on any sample of limited size can always be made to fail provided that it is applied when the mean wastage is sufficiently low. This is a mathematical consequence of the type of distribution which is being considered, and will hold irrespective of the nature of the sample. Conversely, the curve for heterogeneous fruit will always depart from that of homogeneous fruit as wastage is allowed to develop, and will arrive at a stage where it tends to flatten. The

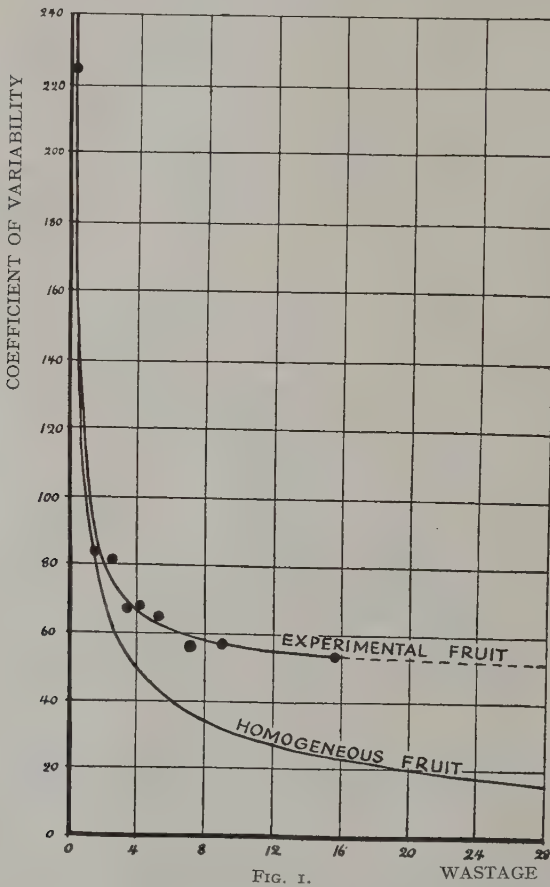

Fig. 1.

time taken for this will vary with the sample; the nearer the approach to homogeneity in the sample, the further the curve will follow the fall of the curve for homogeneous fruit and the longer the tendency towards flattening will be delayed. The tendency for the curve to flatten is due to reaching a stage where the variability of the replicates is due almost entirely to lack of uniformity in the source and treatment of the fruit. The variability which can be ascribed merely to the random scattering of wasty fruits from box to box becomes, at this stage, of small importance. The shape of the curve now depends almost entirely on the experimental conditions; it will be flat so long as experimental conditions remain constant, but will rise or fall if conditions are made less or more uniform. In fact, it may be said that at this stage wastage experiments lose their peculiarity. They become like most other horticultural and agricultural experiments; and the coefficient of variability becomes dependent on, and descriptive of, the experimental conditions and ceases to be greatly influenced by the amount of wastage which is present.

APPLICATION OF RESULTS TO WASTAGE EXPERIMENTS.

From the aspect of their practical application to wastage experiments, the main points of interest about the curve for heterogeneous fruit are :—

1. As with homogeneous fruit, the coefficient of variability of ordinary heterogeneous fruit is very high when the amount of wastage is very low. Samples cannot therefore be easily compared at this stage. Increased wastage causes the coefficient to fall in value and thereby facilitates a comparison of samples. In this respect also heterogeneous fruit behaves like homogeneous fruit. But here the similarity between the two types of fruit ends. Whereas the value of the coefficient for homogeneous fruit continues to fall with increasing wastage, that for heterogeneous fruit soon approaches a minimum and further increases of wastage have only a small effect. With the experimental fruit under consideration the benefit of increasing the wastage almost ceased after only about twelve fruits per box had become wasty. For Fig. 1 shows that coefficient for the experimental fruit falls only 5 per cent. in value as wastage increased from twelve to twenty-eight fruits per box, the corresponding fall for homogeneous fruit being 39 per cent.

2. In most experiments one aims to reduce the variability of the replicates by direct means. In wastage experiments this is done by selecting fruit from a more uniform source and picking, packing, handling and storing it in a more uniform manner. If absolute uniformity is achieved, the fruit becomes homogeneous. Consequently it may be assumed that the homogeneity-value of the coefficient for any given amount of wastage is the minimum value which is possible whatever precautions are taken. The difference between the observed value of the coefficient of variability in any experiment and the corresponding

value for homogeneous fruit is therefore a measure of that fraction of the total variability of the fruit which may be eliminated by the control of experimental conditions.

When the amount of wastage is very low the curves for experimental, heterogeneous fruit and for homogeneous fruit are concurrent. No reduction of the coefficient of variability could therefore be expected from reducing the heterogeneity of samples at this stage, and there is nothing to gain from controlling the uniformity of the experimental conditions.

As wastage develops, the curves diverge and the scope for improving experimental efficiency by reducing the heterogeneity of samples becomes increasingly great.*

The Limitation of the Use of the Coefficient of Variability as a Measure of Efficiency of Wastage Experiments.

The curve for homogeneous fruit in Fig. 1 was based on calculations for boxes containing a total of 150 fruits. The curve covered amounts up to only twenty-eight wasty fruits per box, that is, up to only about 19 per cent. wastage. The boxes used in the experiment on which the curve for heterogeneous fruit was based also contained, on an average, a total of about 150 fruits. Wastage ranged up to about sixteen fruits per box, that is, about 11 per cent., although by extrapolation the curve was extended so as to include proportions up to about 19 per cent.

The curves therefore cover only a small proportion of the total possible wastage, and it is only for these small proportions that the results are intended. The conclusions no longer hold as proportions approaching 100 per cent. are approached. This is due to the fact that the coefficient of variability is really only an approximate measure of experimental efficiency which was used to avoid the more complicated analysis which is necessary for an exact treatment of the problem. The coefficient may be applied with a high degree of accuracy only when the proportion of wastage is small; with increasing wastage it becomes increasingly inaccurate. This is made clear by study of the assumption involved in the application of the coefficient to wastage experiments.

The coefficient of variability is, by definition, the standard deviation as a percentage of the mean. From this it may be concluded that for any given value of the coefficient one would require a definite number of replicates to demonstrate a given *percentage* difference between samples, and that a reduction of the value of the coefficient would always allow a percentage difference to be demonstrated by a smaller number of replicates. These conclusions are accurate and are true under all circumstances. But to apply the coefficient to measure the changes of efficiency which accompany increasing wastage it must be assumed that the percentage difference between samples remains constant throughout the period of storage. It is this assumption which is false. Percentage differences may be expected to decrease as wastage increases, and a comparison of samples will tend to become more difficult even if the value of the coefficient of variability remains constant.

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* To interpret results quantitatively, it should be noted that experimental efficiency varies inversely as the *square* of the coefficient of variability. To take a numerical example from Fig. 1, the coefficient of variability of homogeneous fruit is about a third of that of the heterogeneous, experimental fruit when there are twenty-eight wasty fruits per box. If the heterogeneity of the experimental fruit could have been eliminated by the control of the experimental conditions, the experiment would have become nine times as efficient in the sense that one would have needed only a ninth the number of boxes to demonstrate the same differences between samples.

Consider for simplicity the case of homogeneous fruit. If experimental and biological factors remain constant, the rate at which wastage develops will be proportional to the amount of fruit which still remains sound. If two samples decay at unequal rates, the proportion of sound fruit will be diminished more rapidly in the sample with the faster rate of wastage. In consequence, the retardation of the rate of wastage due to a diminishing reserve of sound fruit will be greater in this sample, and the difference between the samples will tend to be reduced as wastage develops.

This effect is illustrated by some figures in Table I. For the purpose of calculation it is assumed that, were it not for the effect of a diminishing reserve of sound fruit, there would be a difference of 20 per cent. between two samples ; and the table shows how the percentage difference decreases with increasing wastage.

TABLE I.

The Effect of Increasing Wastage on the Percentage Difference between two samples, the initial difference being assumed to be 20 per cent.

Percentage wastage (average of the two samples).	Percentage difference between the samples.
1	20
10	19
20	18
30	17
50	14
70	10
99	1

Decreases are at first small, and, until more than about 20 to 30 per cent. wastage has developed, no very great error would be incurred by assuming that percentage differences between samples are approximately constant.

Similar results are to be expected with heterogeneous fruit.

Over the range of wastage which has been considered it may therefore be taken that the conclusions in the previous sections of this paper are sufficiently true for practical purposes. When, however, the proportion of wastage is higher than about 20 to 30 per cent., the conclusions are no longer justified. This does not imply that the benefits of increasing wastage cease when proportions higher than 30 per cent. are present ; it implies merely that the simplified method of demonstration used is no longer applicable. Actually, increased wastage will be beneficial so long as the fall in the value of the coefficient of variability is greater than the corresponding fall in the percentage difference between samples, but to follow the matter up would involve complex mathematical and biological considerations.

It is fortunate that proportions of wastage higher than 30 per cent. are not often found in commercial practice in export fruit and are consequently of little importance for the type of experiment under consideration. The simplified, approximate method of approaching the problem is therefore probably sufficient.

DISCUSSION.

It has been shown that it is possible to consider even the complex case of heterogeneous fruit on general principles, and the conclusions which have been drawn are applicable to all types of wastage experiment. It is immaterial whether samples are compared by measuring the amount of wastage developed after a given period of storage or by measuring the time taken for a given amount of wastage to develop. It is also immaterial whether boxes of fruit are used as replicates or whether some other system of replication is adopted. The application of the conclusions to any given type of experiment require only an appropriate modification of detail, and it is not intended here to discuss any special case.

The results show that measures for the improvement of experimental efficiency must be applied in two separate directions according to the circumstances. If only a small amount of wastage is expected in an experiment, efforts should be concentrated on securing more wastage. The reduction of the heterogeneity of the samples by selecting more uniform fruit and handling it in a more uniform manner is of little use at this stage. When large amounts of wastage are expected the position is reversed ; efforts should be concentrated on reducing the heterogeneity of the samples, and little is to be gained from increasing the amount of wastage.

Difficulties may be expected in raising the amount of wastage to a level which can be regarded as experimentally efficient. The direct method of prolonging the storage period is not always sound, since the type of wastage found after extended storage is often different from that developed under ordinary commercial conditions. An investigation of the possibility of using other methods (involving, for example, the dusting of fruits with the spores of the fungus concerned) is therefore urgently needed.

SUMMARY.

The investigation is concerned with the factors which influence the magnitude of the standard error of estimates of wastage in samples of fruit which are stored for a limited period comparable with that used in commercial practice to bring the fruit from the grower to the consumer. The problem is complicated by the fact that the distribution of wastage among replicates is discontinuous, as a result of which :

1. The percentage standard error is greatly influenced by the amount of wastage developed in the sample.

2. When only a small amount of wastage has developed, the percentage standard error always tends to be high, and a comparison of the wastage in samples is consequently difficult.

3. As wastage increases, the percentage standard error decreases, at first rapidly but later more slowly until finally it approaches a minimum and further increases in wastage have scarcely any effect. When only a small amount of wastage is anticipated in an experiment, a large gain in experimental efficiency may therefore be expected from measures designed to increase wastage. When, however, a large amount of wastage is anticipated, there is little purpose in attempting further to increase the wastage.

4. The selection of more uniform fruit, its handling under more uniform conditions and other measures designed to make samples less heterogeneous are not effective in reducing the standard error when only a small amount of wastage is present. Such measures should be used only in experiments in which a large amount of wastage is expected.

These conclusions have been reached with the aid of the simplifying assumption that the coefficient of variability (i.e. the percentage standard deviation) is a measure of experimental efficiency. This assumption becomes inaccurate when the proportion of wastage is very high (above about 30 per cent.). The conclusions are therefore not intended to apply at this stage.

REFERENCES.

(1) *Putterill, V. A.* and *Davies, Rees.* Citrus Wastage Investigations carried out at Zebediela, Transvaal, during the Seasons 1931 and 1932. Union of S. Africa, Dept. of Agric., Bull. 128, 1934.

(2) *Hoblyn, T. N.* Field Experiments in Horticulture. Imp. Bur. Fruit Prod., T.C.2, 1931.

CHANGES IN THE CHEMICAL COMPOSITION OF DEVELOPING APPLES

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INTRODUCTION.

During the 1934–5 season the opportunity was presented of recording the rate of development and the changes in chemical composition undergone by Dunn's Favourite and Jonathan apples on two orchards in the Nelson Province, New Zealand. As there is little information in the literature tracing the growth of apples from the young stage to approximate maturity it was considered of interest to bring the data together and to examine them in the light of the results of other workers in this field of fruit research.

I. EXPERIMENTAL.

Observations of the growth of the two varieties already mentioned have been made on orchards at Braeburn and Mildura, about five miles distant by direct line from one another and located on the Moutere Hills soil type. This soil type is derived from an ancient river system which deposited a mixture of soil-forming gravels, sands and silts. The soil type covers about 400 square miles but only the low hill slopes on the north-eastern side of this area are used for fruit-growing. The depth of soil varies somewhat as does the quality of the soil; in all cases however marked lime and phosphate deficiencies are present so that very good crop responses are obtained from the use of limestone and superphosphate. On the areas from which the present series of apple samples were obtained a system of fertilization employing nitrogen, phosphorus and potassium has been employed. The development of the trees has in all cases been satisfactory for this type of soil.

Throughout the season at approximately weekly intervals, commencing on November 12th, 1934, about thirty-five days after the maximum blossom, samples of fruit were collected from the two varieties, Dunn's Favourite and Jonathan, on each of the orchards. Sufficient fruits were collected each time to allow the determination of moisture, total nitrogen and total ash, as well as to permit the staining with iodine for indication of starch formation and to permit a reliable estimate of the average weight and diameter of the fruits.

The moisture was estimated by drying thin equatorial slices of fruit, including skin and core, after moistening with alcohol, for twenty hours at 103° C. Nitrogen was estimated by the usual Kjeldahl method and ash by ignition at about 450° C. Some difficulty was experienced in the nitrogen estimations, especially in the larger apples owing to slight variations in the weight of pips included. Some of the irregularities in the nitrogen figures in the tables which follow are probably due to this cause. For a similar reason there are some irregularities in the ash contents towards the end of the experimental period.

The average weight was determined using from thirty to thirty-five apples, the stem being removed; the average diameter was obtained from twenty-five to thirty fruits whose diameters in two directions at right angles were measured.

Data on the rainfall during the season are included. The figures refer to the Government Research Orchard at Appleby, a few miles to the east of the orchards under examination in the present experiments. The Braeburn orchard probably would receive the greatest precipitation with Mildura next and the Research orchard least.

TESTS ON EXPERIMENTAL PROCEDURE.

In the determination of the moisture and total ash slices 1 to 2 mm. in thickness were taken equatorially through the apples. For small apples the whole slices were used, but for the large apples opposite quadrants of the slices were used in duplicate estimations. The justification for this procedure is shown in Table I. In this experiment, using large Jonathan fruits, horizontal slices were taken through ten apples at five points.

TABLE I.

Moisture and Ash Contents of Different Sections of Jonathan Apple, Mildura.

Expressed as Percentage of Fresh Material.

Section.	Moisture per cent.	Ash per cent.
I	86·19	0·218
II	86·11	0·188
III	85·46	0·223
IV	85·78	0·266
V	84·91	0·281
Mean	85·69	0·235

Section I	Through apple just below bottom of stem.
Section II	Bottom of slice just below bottom of carpels.
Section III	Median section through carpels (no pips included).
Section IV	Taken just above the carpels.
Section V	Taken as near as possible to bottom of calyx opening.

From the data from the moisture and ash contents there appears to be a decrease in moisture content and an increase in ash content in passing from the stem end to the calyx end of the fruit; the mean values for the moisture and ash are seen to correspond satisfactorily with those found for the equatorial slice (section 3). The inclusion of pips in the samples used in the main series of experiments would decrease slightly the moisture content and increase slightly the ash content of the equatorial slices used in succeeding estimations.

An experimental error test for the moisture and ash determinations was carried out using eight samples of ten apples each. From the data reported in Table II it will be seen that in general the duplicate determinations with any one sample agreed better than the values obtained for any two samples. Sample V contained some badly russetted fruit, which may explain the comparatively large percentage differences in the constituents from those found in the other cases. Excluding the data for this sample the probable errors for a single determination of the moisture and ash contents have been calculated. They are for moisture 0·28 per cent. and for ash 0·009 per cent. These values may be considered satisfactorily low.

TABLE II.

Experimental Error Test for Moisture and Ash Contents, Jonathan Apple, Mildura.

Expressed as Percentage of Fresh Material.

Sample.	Moisture per cent.		Ash per cent.	
	I	II	I	II
I	82·55	82·45	0·295	0·270
II	83·06	82·87	0·288	0·293
III	83·00	82·64	0·294	0·281
IV	81·90	82·61	0·282	0·250
V*	83·89	83·88	0·270	0·277
VI	83·00	83·19	0·297	0·294
VII	83·49	82·95	0·281	0·297
VIII	83·37	83·13	0·293	0·306

Nos. V-VIII dried on different day in oven from Nos. I-IV, but all were weighed out on same day.

Duplicate moisture and ash on each sample by taking opposite quarters of each slice for a given sample; pips were included.

* Badly russetted fruit in this sample; not included in calculation of probable error.

II. RATE OF GROWTH OF FRUITS.

The data in Tables III to VI show the manner in which the fruits increased in weight and size. When the first samples were taken the Dunn's Favourite averaged on both orchards about 5 grm. in weight and 20 mm. in diameter, while the Jonathan in one orchard averaged 5·55 grm. and 22·1 mm., and in the other 3·36 grm. and 18·1 mm. respectively. As the season progressed there was a tendency for the Mildura Dunn's to grow more rapidly than the Braeburn fruit. Jonathan fruit from Mildura behaved in a similar though less marked manner.

When further examination of the sets of figures is made it becomes evident that there is a distinct tendency for the fruit to increase in weight and diameter after heavy rain and for a slight slowing down of growth during relatively dry periods. For example heavy rain was experienced over the period December 17th, 1934, to January 7th, 1935, and during this period relatively great daily average increases in size are found. Rains towards the end of January and the end of February affected the fruit in a similar manner.

It is quite clear also that the fruit had by no means ceased growing when the last samples were collected. Commercial pickings were made at the end of February, enforcing the cessation of experimental work.

TABLE III.

Rate of Growth and Chemical Composition of Dunn's Apple, Braeburn.

Date of Sampling.	Rainfall Inches.	Moisture per cent.	Dry Matter per cent.	Total Nitrogen per cent.	Total Ash per cent.	Average Weight (grm.).	Average Diameter (mm.).
12/11/34	Trace	88·11	11·89	0·254	0·463	5·27	20·6
20/11/34	1·41	89·50	10·50	0·184	0·349	9·85	26·5
26/11/34	0·22	88·38	11·62	0·168	0·347	14·45	31·9
3/12/34	Nil	88·32	11·68	0·162	0·283	20·8	35·8
10/12/34	Nil	87·14	12·86	0·149	0·291	28·3	41·0
17/12/34	0·06	85·47	14·53	0·156	0·330	31·5	44·0
26/12/34	3·19	85·93	14·07	0·124	0·241	58·8	51·0
7/ 1/35		85·51	14·49	0·099	0·245	76·1	55·2
14/ 1/35	Trace	85·63	14·37	0·103	0·249	86·1	57·8
21/ 1/35	1·42	84·67	15·33	0·086	0·196	94·4	60·7
28/ 1/35	0·19	84·92	15·08	0·095	0·203	112·7	63·8
4/ 2/35	0·33	85·14	14·86	0·091	0·170	115·0	65·2
11/ 2/35	0·12	85·17	14·83	0·058	0·166	128·0	68·0
18/ 2/35	0·08	84·55	15·45	0·085	0·162	142·8	69·1
25/ 2/35	2·61	86·16	13·84	0·064	0·163	157·6	72·1

TABLE IV.

Rate of Growth and Chemical Composition of Dunn's Apple, Mildura.

Date of Sampling.	Rainfall Inches.	Moisture per cent.	Dry Matter per cent.	Total Nitrogen per cent.	Total Ash per cent.	Average Weight (grm.).	Average Diameter (mm.).
12/11/34	Trace	88·55	11·45	0·289	0·611	4·75	20·3
20/11/34	1·41	90·05	9·95	0·229	0·456	10·21	27·7
26/11/34	0·22	89·25	10·75	0·204	0·536	14·2	31·5
3/12/34	Nil	88·45	11·55	0·170	0·414	22·6	37·2
10/12/34	Nil	87·62	12·38	0·160	0·391	32·7	42·0
17/12/34	0·06	87·38	12·62	0·147	0·353	42·3	46·0
26/12/34	3·19	87·29	12·71	0·126	0·344	61·4	52·5
7/ 1/35		87·07	12·93	0·087	0·312	88·4	58·4
14/ 1/35	Trace	86·68	13·32	0·091	0·311	105·6	62·6
21/ 1/35	1·42	86·49	13·51	0·090	0·262	113·6	64·5
28/ 1/35	0·19	86·30	13·70	0·106	0·285	120·9	67·2
4/ 2/35	0·33	86·87	13·13	0·080	0·245	121·9	66·7
11/ 2/35	0·12	86·87	13·13	0·094	0·244	141·1	69·6
18/ 2/35	0·08	87·26	12·74	0·094	0·211	155·0	72·6
25/ 2/35	2·61	88·04	11·96	0·065	0·216	175·4	75·2

TABLE V.

Rate of Growth and Chemical Composition of Jonathan Apple, Braeburn.

Date of Sampling.	Rainfall Inches	Moisture per cent.	Dry Matter per cent.	Total Nitrogen per cent.	Total Ash per cent.	Average Weight (grm.).	Average Diameter (mm.).
12/11/34	Trace	88·47	11·53	0·222	0·498	5·55	22·1
20/11/34	1·41	89·66	10·34	0·169	0·388	9·99	27·2
26/11/34	0·22	88·10	11·90	0·162	0·469	11·60	29·0
3/12/34	Nil	87·50	12·50	0·159	0·356	17·5	33·9
10/12/34	Nil	86·00	14·00	0·143	0·348	22·9	38·0
17/12/34	0·06	85·47	14·53	0·156	0·330	31·5	41·0
26/12/34	3·19	85·09	14·91	0·138	0·315	46·5	47·4
7/ 1/35		84·61	15·39	0·085	0·312	65·6	53·3
14/ 1/35	Trace	84·95	15·05	0·094	0·319	74·9	55·2
21/ 1/35	1·42	83·50	16·50	0·096	0·240	81·3	57·2
28/ 1/35	0·19	82·55	16·45	0·092	0·279	98·5	61·5
4/ 2/35	0·33	82·91	17·09	0·080	0·226	104·7	63·2
11/ 2/35	0·12	83·37	16·63	0·048	0·208	122·7	66·4
18/ 2/35	0·08	83·14	16·86	0·070	0·206	131·8	67·8
25/ 2/35	2·61	84·23	15·77	0·053	0·242	150·0	70·2

TABLE VI.

Rate of Growth and Chemical Composition of Jonathan Apple, Mildura.

Date of Sampling.	Rainfall Inches.	Moisture per cent.	Dry Matter per cent.	Total Nitrogen per cent.	Total Ash per cent.	Average Weight (grm.).	Average Diameter (mm.).
12/11/34	Trace.	87·92	12·08	0·246	0·542	3·36	18·1
20/11/34	1·41	89·23	10·77	0·192	0·420	7·35	23·3
26/11/34	0·22	88·05	11·95	0·179	0·479	8·05	24·0
3/12/34	Nil	87·61	12·39	0·143	0·362	9·26	30·2
10/12/34	Nil	86·83	13·17	0·126	0·356	20·1	36·0
17/12/34	0·06	86·06	13·94	0·125	0·300	29·3	41·0
26/12/34	3·19	84·78	15·22	0·101	0·294	45·6	47·7
7/ 1/35		84·22	15·78	0·055	0·293	67·2	52·8
14/ 1/35	Trace	84·72	15·28	0·061	0·290	75·1	55·5
21/ 1/35	1·42	83·41	16·59	0·056	0·251	90·2	58·8
28/ 1/35	0·19	82·57	17·43	0·066	0·257	100·9	62·5
4/ 2/35	0·33	83·24	16·76	0·063	0·239	105·3	63·3
11/ 2/35	0·12	82·68	17·32	0·047	0·242	121·3	67·3
18/ 2/35	0·08	83·82	16·18	0·070	0·207	133·5	67·9
25/ 2/35	2·61	84·57	15·43	0·044	0·220	156·7	71·7

III. CHEMICAL COMPOSITION OF FRUITS.

The chemical data shown in Tables III to VI indicate that the percentages of total nitrogen and of total ash are at relatively high levels at the beginning of the season and that they fall fairly steadily to approximately constant values with increasing size of fruit. At the second sampling the ash content of all the samples, except the Braeburn Dunn's, shows a minimum figure which may be connected with the relatively high moisture content found at that time. In the cases of both the nitrogen and the ash contents the samples from Mildura show the higher values for both varieties of apple, the Dunn's Favourite, however, being somewhat higher than the Jonathan variety.

The moisture content, except for the second sample, tends to fall as the fruit develops, but after heavy rains this fall is arrested, as may be seen from the data covering the period December 17th to January 14th. The heavy rain at the end of February has definitely increased the moisture content of the fruit of all four samples.

The growth, status and size of the crop carried by the trees in the two orchards must affect fruit development and moisture content. Certain differences, more marked in the Dunn's Favourite samples, in the variation of the moisture contents throughout the season of fruit from the two orchards are probably due to these causes.

Attention may be drawn to the minimum values of dry matter found for the samples collected on November 20th. This appears to be a natural effect since the data of Archbold (2) for another variety of apple indicate a similar minimum dry matter content for young fruits at a certain stage of their growth. It would therefore seem that this minimum is found independently of the variety under examination.

IV. DEVELOPMENT OF STARCH IN APPLES.

Archbold (2) has shown by chemical methods that starch does not appear in certain varieties of apple until the fruit is approximately twenty-one days old. It was of interest therefore to see when starch appeared in the fruit examined in these experiments. Staining of the starch granules with iodine was the method adopted for showing the presence of starch in the fruit.

In the first samples, collected on November 12th, starch was just making its appearance although the fruits were approximately thirty-five days old. The Mildura Dunn's Favourite collected on this date are shown in Fig. (*a*) of Plate I. The Jonathan fruit from this orchard on the same date gave no sign of the presence of starch. The fruit from the Braeburn orchard showed the merest trace of starch in the narrow band near the periphery of the fruit. As the fruit grows this band increases in width as shown in Fig. (*b*) of Plate I, until finally the staining indicates that starch has appeared within the core area.

When the fruit is approaching maturity the starch begins to disappear from the core area first, as shown in Fig. (*a*) of Plate II, and with increasing degree of maturity this clear area widens until finally most of the flesh shows the absence of starch (Fig. (*b*), Plate II).

It is interesting that the presence of starch should be first noticed near to the periphery of the flesh and that it should remain in this area until the last. Even in the last samples collected for these experiments much starch remained in the fruits indicating that the rate of hydrolysis had not overtaken the rate of formation of starch in the outer areas of the flesh.

IV. DISCUSSION.

Before passing on to the discussion of the uptake of solids by the fruit during growth it is desirable to draw attention to the fact that the average weights for the fruits when plotted against age in days do not conform to the conclusion reached by Archbold (2) that after the appearance of starch in the apple the increase in weight progresses linearly with age of the fruit. In the present series starch was just beginning to appear about the time of taking the first samples but the growth curves show very distinct curvature for a period of some weeks after this date. Throughout the remainder of the season even it can hardly be said that the weight varies in a linear way with the age of the fruit. Differences of soil conditions, nutrition, and size of crop must affect the rate of growth. Indeed the weight figures in the tables already discussed show this distinctly; for example, after the middle of December the Dunn's Favourite apples at Mildura were consistently larger than apples at Braeburn on corresponding dates, although at the time of taking the first sample the fruits from the two orchards were identical in size. Another interesting point concerns the relation between the diameter and weight

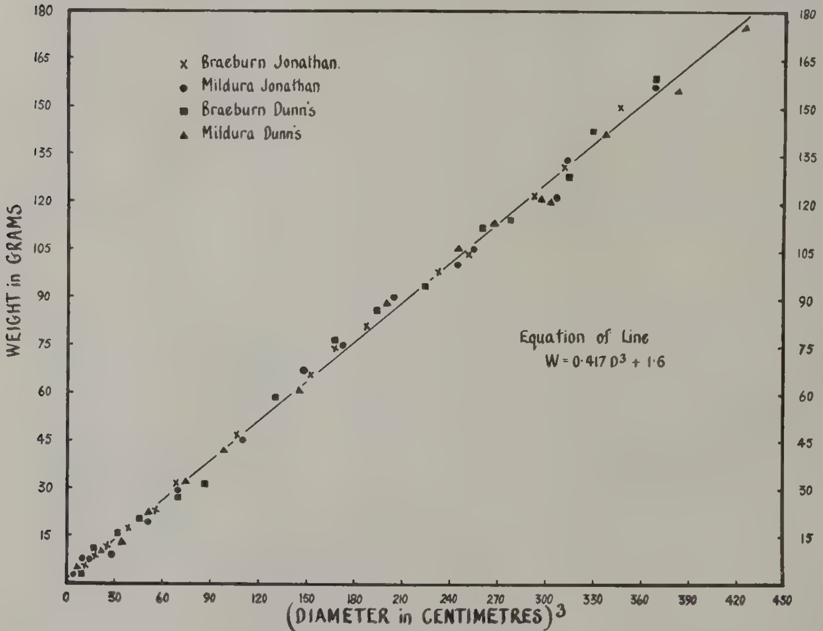

FIG. 1.

Showing linear relation between weight in grams and cube of the diameter (in centimetres) for Jonathan and Dunn's Favourite fruits from two orchards.

of the fruits at different stages of growth. In Fig. 1 are plotted the average weights for the four sets of fruits against the cube of the diameter, measured in centimetres. It is seen that all the points lie very closely on the same line. Such deviations as are shown may very easily be due to irregularity of sampling or simply that the cube of the diameter is not a correct measure of the volume of the fruit. This latter possibility would be expected to operate to greater effect in the case of the Dunn's Favourite which is by no means a round apple. The constant, 1·6, which appears in the equation of this line may be connected with the deviation of the shape of the apple from a sphere. Variation from a spherical shape cannot explain all the deviations from the given line as these are evenly distributed between the Jonathan and the Dunn's Favourite. Now, if the fruits were spheres, the apparent density would be given by the equation $W = 0 \cdot 5237\, d\, D^3$

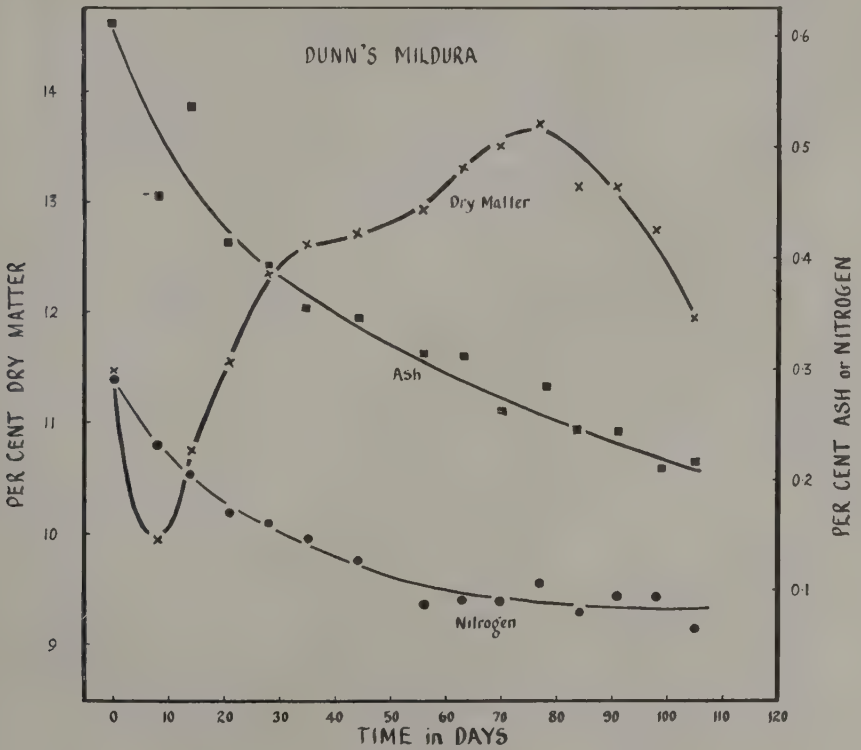

FIG. 2.

Showing percentage of dry matter, nitrogen and ash of Dunn's Favourite fruits, Mildura, throughout the experimental period.

where d is the apparent density, W the weight in grams, and D the diameter in centimetres. But from the equation of the line in Fig. 1 the relation is $W = 0{\cdot}417D^3 + 1{\cdot}6$ where W and D have the same significance as in the last equation. So that where W is large compared with 1·6, d is equal to 0·417/0·5237 or 0·796. It would appear therefore that except in the case of small apples the apparent density of these two varieties lies between 0·79 and 0·80 and does not show any great variation over a considerable portion of the season.

In Fig. 2 are set out graphically the percentages of dry matter, nitrogen and ash for Dunn's Favourite from the Mildura orchard. In all cases the percentages vary considerably throughout the season, the percentage of dry matter tending to increase with increase in size of the fruit, while, over most of the season, the nitrogen and ash percentages decrease in a regular manner. Each of the other three sets of samples gives data leading to a similar conclusion. It has been suggested above that some of the variations in the dry matter percentages can be generally correlated by rainfall; but it does not appear that rainfall has had such marked influences on the nitrogen and ash percentages.

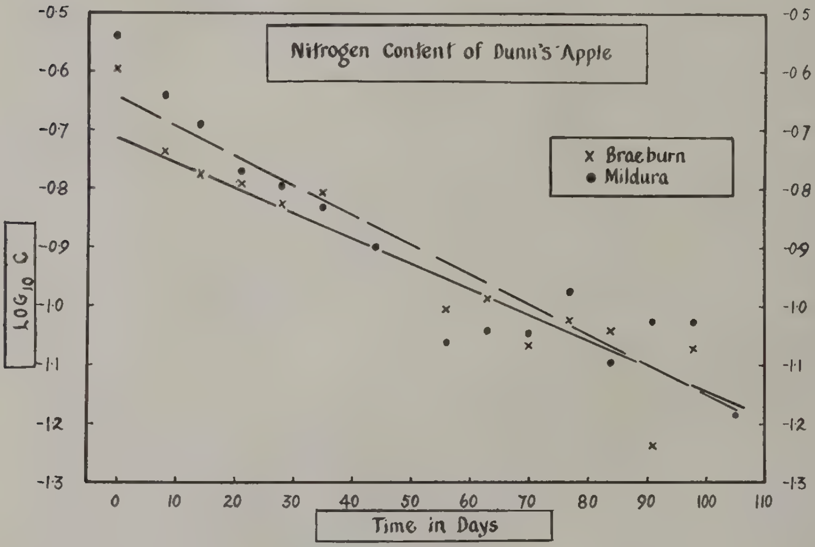

FIG. 3.

Showing logarithmic relation between nitrogen percentage and age of Dunn's Favourite fruits. Lines of closest fit calculated by method of least squares.

It has been shown by several workers that the concentration of certain constituents of fruits decreases with age according to an exponential law. The present data for the nitrogen and ash contents have been examined with this in mind. The Dunn's Favourite from Mildura and Braeburn have been selected for graphical representation as typical of the results obtained. Figures 3 and 4 show respectively the graphs for the nitrogen and ash percentages when the logarithm of the concentration of the constituent is plotted against the age of the apple. It will be seen that, except for very small apples and for the largest apples, when difficulties of estimation of the constituents, nitrogen in particular, are encountered, the points lie on straight lines.

By the method of least squares lines of closest fit may be calculated* for the two constituents and for the four sets of observations. The equations to these lines are as follows, where C is the percentage of constituent and t the time in days from the date of the first sampling.

SAMPLE.	EQUATION.
For Nitrogen :	
Dunn's Braeburn	$\log_{10} C = -0{\cdot}713 - 0{\cdot}00432\,t.$
Dunn's Mildura	$\log_{10} C = -0{\cdot}643 - 0{\cdot}00508\,t.$
Jonathan Braeburn	$\log_{10} C = -0{\cdot}703 - 0{\cdot}00502\,t.$
Jonathan Mildura	$\log_{10} C = -0{\cdot}737 - 0{\cdot}00590\,t.$
For Ash :	
Dunn's Braeburn	$\log_{10} C = -0{\cdot}395 - 0{\cdot}00400\,t.$
Dunn's Mildura	$\log_{10} C = -0{\cdot}297 - 0{\cdot}00359\,t.$
Jonathan Braeburn	$\log_{10} C = -0{\cdot}362 - 0{\cdot}00295\,t.$
Jonathan Mildura	$\log_{10} C = -0{\cdot}363 - 0{\cdot}00298\,t.$

The equations are of the form $\log C = a + bt$, where a shows the concentration at the beginning of the experiment and b the daily rate at which the logarithm of the concentration decreases with the increase in age of the fruit.

However, because the points for the first date of sampling appear to lie off the lines passing through the other points and have been omitted in the calculations of the lines of closest fit, it is not permissible to extrapolate to zero time; or in other words the values of the constant a cannot be used as a measure of the original concentration of ash or nitrogen.

After ten days from the beginning of the experiment the values in the above equations may be used to calculate the percentages of ash or nitrogen for the different samples.

The calculated values at zero time are, however, sufficiently in accord with the analytical data to indicate that the Dunn's samples from Mildura have a distinctly greater nitrogen and ash content than the corresponding Braeburn ones. The rate of decrease of the nitrogen content is greater at Mildura than at Braeburn, but the ash content decreases rather more slowly at Mildura than at Braeburn. For the Jonathan samples the original nitrogen contents are relatively close but the rate of decrease with age is distinctly greater at Mildura; in the case of the ash contents the percentages and rates of decrease with age are almost identical for the two sets of samples.

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* Observations for the first sample in each series are excluded.

It is of interest to note that the Dunn's samples tend to show higher nitrogen contents than the Jonathan samples, and, except for the higher figure for the Mildura Dunn's ash content, the early ash contents of the samples are similar in magnitude.

Whether the variations in the rates of fall of the concentrations of the constituents for the several sets of samples reflect differences in the nitrogen status of the trees and soil cannot be stated with certainty owing to the scattering of the points towards the end of the season. The great difference between the rates of decrease in the ash contents of the two varieties would appear to be a true varietal effect.

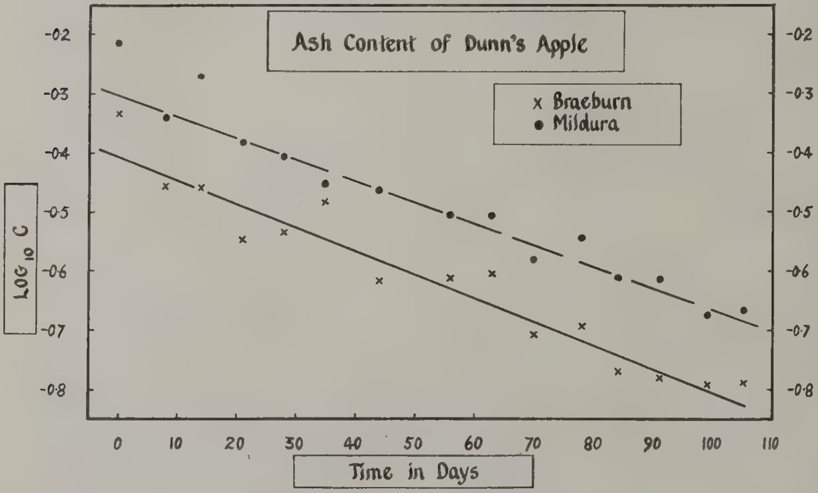


FIG. 4.
Showing logarithmic relation between ash percentage and age of Dunn's Favourite fruits. Lines of closest fit calculated by method of least squares.

It is therefore clear that the nitrogen and ash percentages decrease with age of the fruit according to an exponential law of a form similar to that found by other workers for constituents such as acid or sugars, and for different experimental conditions.

Although the weight curves and the percentage composition curves throw considerable light on the growth of the fruits, yet they do not tell the whole story: from the point of view of nutrition it is of importance to know the rate at which the various constituents such as dry matter, total nitrogen and total ash are being taken up. The full data for these three constituents are set out in Tables VII to X; in Fig. 5 are set out in graphical form the data of Table VIII, this set of observations being taken as typical of those obtained.

TABLE VII.

Growth of Dunn's Apple—Braeburn 1934-5 *Season.*

Uptake of Dry Matter, Total Nitrogen and Total Ash per Fruit in grams.

Date of Sampling.	Dry Matter.	Nitrogen.	Ash.
12/11/34	0·63	0·0134	0·0244
20/11/34	1·03	0·0181	0·0344
26/11/34	1·68	0·0243	0·0501
3/12/34	2·43	0·0337	0·0589
10/12/34	3·64	0·0422	0·0823
17/12/34	4·58	0·0491	0·1039
26/12/34	8·27	0·0729	0·1417
7/ 1/35	11·10	0·0758	0·1877
14/ 1/35	12·37	0·0887	0·2144
21/ 1/35	14·47	0·0812	0·1850
28/ 1/35	17·00	0·1071	0·2288
4/ 2/35	17·09	0·1047	0·1955
11/ 2/35	18·98	0·0742	0·2125
18/ 2/35	22·06	0·1214	0·2313
25/ 2/35	21·81	0·1009	0·2569

TABLE VIII.

Growth of Dunn's Apple—Mildura 1934-5 *Season.*

Uptake of Dry Matter, Total Nitrogen and Total Ash per Fruit in grams.

Date of Sampling.	Dry Matter.	Nitrogen.	Ash.
12/11/34	0·54	0·0137	0·0290
20/11/34	1·02	0·0234	0·0466
26/11/34	1·53	0·0290	0·0761
3/12/34	2·61	0·0384	0·0936
10/12/34	4·05	0·0523	0·1278
17/12/34	5·34	0·0622	0·1493
26/12/34	7·80	0·0774	0·2112
7/ 1/35	11·43	0·0769	0·2758
14/ 1/35	14·07	0·0961	0·3284
21/ 1/35	15·35	0·1022	0·2976
28/ 1/35	16·56	0·1281	0·3446
4/ 2/35	16·00	0·0975	0·2986
11/ 2/35	18·53	0·1326	0·3443
18/ 2/35	19·75	0·1457	0·3270
25/ 2/35	20·98	0·1140	0·3789

TABLE IX.

Growth of Jonathan Apple—Braeburn 1934-5 *Season.*

Uptake of Dry Matter, Total Nitrogen and Total Ash per Fruit in grams.

Date of Sampling.	Dry Matter.	Nitrogen.	Ash.
12/11/34	0·64	0·0123	0·0276
20/11/34	1·03	0·0169	0·0388
26/11/34	1·38	0·0188	0·0544
3/12/34	2·19	0·0278	0·0623
10/12/34	3·21	0·0327	0·0797
17/12/34	4·58	0·0491	0·1040
26/12/34	6·93	0·0642	0·1465
7/ 1/35	10·10	0·0558	0·2047
14/ 1/35	11·27	0·0704	0·2389
21/ 1/35	13·41	0·0780	0·1951
28/ 1/35	16·20	0·0906	0·2748
4/ 2/35	17·89	0·0838	0·2366
11/ 2/35	20·40	0·0589	0·2552
18/ 2/35	22·22	0·0923	0·2715
25/ 2/35	23·66	0·0795	0·3630

TABLE X.

Growth of Jonathan Apple—Mildura 1934-5 *Season.*

Uptake of Dry Matter, Total Nitrogen and Total Ash per Fruit in grams.

Date of Sampling.	Dry Matter.	Nitrogen.	Ash.
12/11/34	0·41	0·0083	0·0182
20/11/34	0·79	0·0141	0·0309
26/11/34	0·96	0·0144	0·0386
3/12/34	1·15	0·0132	0·0335
10/12/34	2·65	0·0253	0·0716
17/12/34	4·08	0·0366	0·0879
26/12/34	6·94	0·0461	0·1341
7/ 1/35	10·60	0·0370	0·1969
14/ 1/35	11·48	0·0458	0·2178
21/ 1/35	14·96	0·0505	0·2264
28/ 1/35	17·59	0·0666	0·2593
4/ 2/35	17·65	0·0663	0·2517
11/ 2/35	21·01	0·0570	0·2935
18/ 2/35	21·60	0·0934	0·2763
25/ 2/35	24·18	0·0689	0·3447

Taking first the dry matter it is seen from Fig. 5 that the total solids increase rapidly in the young fruit, but that after about ninety-five days from blossoming the rate of accretion slows down somewhat, but yet remains relatively great. As has been pointed out above the apples were still increasing in weight when the series of observations was concluded. The rate of accretion of solids is therefore by no means constant. It is of interest to note that the absolute increase is over forty-fold, being from 0·5 grm. to 21 grm.

The data for the nitrogen and ash intake show also that after about 95 days from blossoming the rate of uptake of these constituents shows a distinct decrease in each case, and that after about 120 days there is little increase in the uptake of these constituents. This observation is only partly in keeping with that of Archbold (1) who concluded, in the case of Bramley's Seedling apple, that most of the nitrogen was stored during the first seven weeks of the life of the apple. In the present series, however, the intake of nitrogen has proceeded over a much longer period before any marked decrease in the rate of intake is shown. Some allowance in the length of this period must of course be made for the individuality of the variety of the apple and for its conditions of growth. It is quite clear from the directions of the curves for the nitrogen and the ash percentages that an approximately constant figure is being approached in each case. But the amounts of these constituents taken up over the experimental period increase at least ten-fold from the first samples to the final samples, the weights per fruit rising from about 14 mg. of nitrogen and 29 mg. of ash to 140 mg. and 360 mg. respectively. Excluding the final data for the nitrogen and ash contents, for reasons already

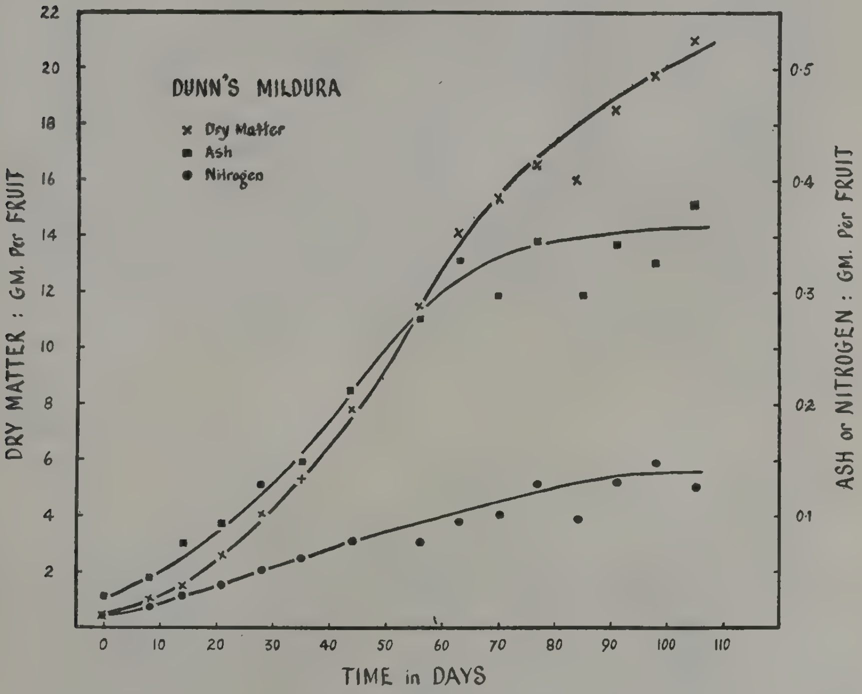


FIG. 5.
Showing accretion of dry matter, and intake of nitrogen and ash for Dunn's Favourite fruits, Mildura, over the experimental period.

suggested, it is evident that the three constituents shown in Fig. V are taken up in a very regular manner.

SUMMARY.

1. Apples of Jonathan and Dunn's Favourite varieties have been examined in two orchards on the Moutere Hills soil type of the Nelson Province.

2. Throughout the season all the fruits grew satisfactorily. Some differences in the rates of growth in the two orchards were noted for samples collected weekly throughout the season.

3. There was some indication that heavy rain was followed by increase in the rate of growth and in the moisture content of the fruit.

4. By staining the starch granules in the flesh of the fruit with iodine it was shown that in the first sample traces only of starch were present. Starch formation begins in the layers of tissue near the periphery and progresses towards the core area. With maturing fruit the core areas are the first to be cleared of starch; the last starch to be hydrolysed is that contained in cells nearer the periphery of the fruit.

5. The nitrogen and ash percentages decreased steadily throughout the experimental period according to the equation $\log_{10}C = a + bt$ where C was the concentration of the constituent concerned, a and b were constants and t was the time in days from the taking of the first sample. The initial concentrations and the rates of fall of these constituents varied with the variety and with the growth conditions of the orchard.

6. The rate of accretion of dry matter, and of intake of nitrogen and ash decreased after about 95 days from the date of maximum blossom, slightly in the cases of dry matter and greatly in the cases of nitrogen and ash. After about 120 days the uptake of nitrogen and ash apparently ceased.

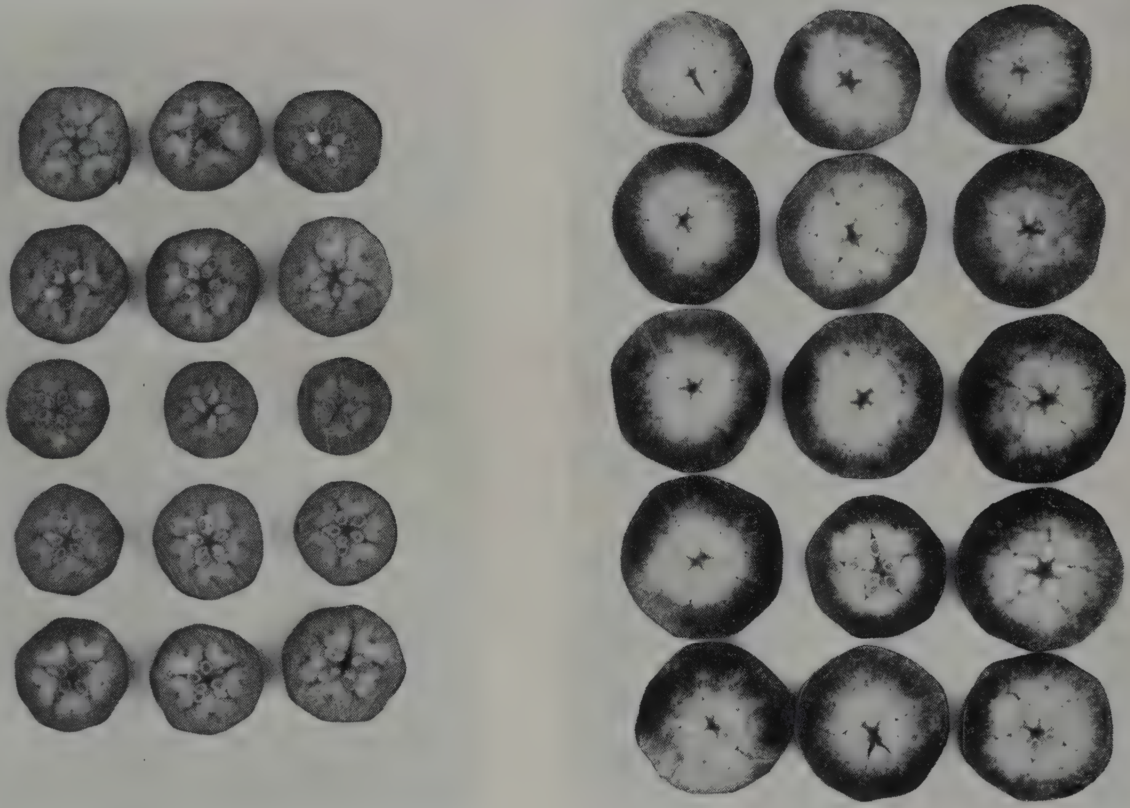
ACKNOWLEDGMENTS.

The author wishes to thank Messrs. E. Chittenden and O. Barton for collecting samples; Miss E. B. Kidson and Mr. D. J. Stanton for assistance with the laboratory determinations; and Mr. O. B. Pemberton for supplying the rainfall data.

REFERENCES.

(1) *Archbold, H. K.* (1928). Chemical Studies in the Physiology of Apples, IX. Ann. Bot. Vol. XLII, p. 541.

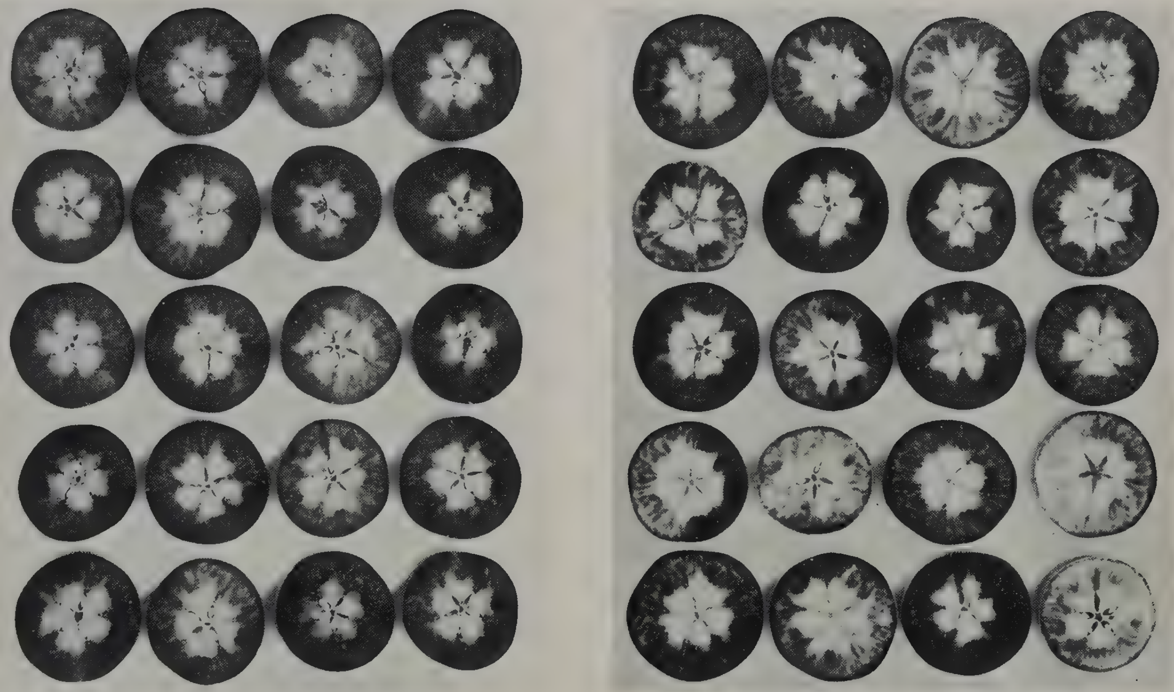
(2) ———————— (1932). Chemical Studies in the Physiology of Apples, XII. Ann. Bot. Vol. XLVI, p. 407.



(*a*) (*b*)

PLATE I.

Iodine Staining of young Dunn's Favourite apples. Note formation of starch in the tissue near periphery of the fruit. Sampled (*a*) on November 12th and (*b*) on November 26th, 1934.



(*a*)

(*b*)

Plate II.

Iodine staining of maturing Dunn's Favourite apples. Note disappearance of starch from core area and presence of starch nearer the periphery of the fruit. Sampled (*a*) on February 11th and (*b*) on March 4th, 1935.

ON THE BOTRYTIS DISEASE OF LETTUCE, WITH SPECIAL REFERENCE TO ITS CONTROL

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THE earlier phases of the work on lettuce diseases which was begun by the writer in 1930 and which was centred at the Biological Field Station, Slough, were carried out by Abdel-Salam, an account of whose results has been published (Journ. Pom. & Hort. Sci., 1934, p. 15, vol. 12). Abdel-Salam surveyed the occurrence of the *Botrytis* disease on commercial plantings over the whole season and found that, while the fungus attacks lettuce plants to some extent at all times of year, both under glass and out of doors, the economically important occurrence was on lettuce seedlings overwintered in cold frames. This method of cultivation is practised on a considerable scale in the neighbourhood of London and elsewhere, and its object is to produce seedlings which are fairly advanced in early spring for planting out in the field at the earliest practicable moment, say in February and March. By so doing the grower is able to forestall the bulk of the summer crop which derives from seed drilled in the open.

The method of frame cultivation leads to considerable risk of damage by fungi, more particularly by *Botrytis*. To combat this the grower attempts to keep the plants as dry as possible. The lights of the frames are kept on at night and during rain, and are taken off only in dry weather. To obtain the maximum of light and warmth, the soil on which the frames are built is steeply sloped to the south, a procedure which has the further advantage of keeping soil moisture at a minimum. Nevertheless, in spite of all these precautions, the disease is apt to appear, more particularly in December and January when light is poor and when spells of cold humid weather are liable to occur. These conditions, so favourable to fungal attack, are accentuated by the crowding of the seedlings in the frames and this is all the greater if the early winter has been mild, with resultant good growth of the plants. It is extremely common therefore to find frames which show patches of dead and dying plants as time goes on, the affected plants bearing abundant conidiophores of *Botrytis*. At the time of transplanting into the field, these bare patches may have seriously reduced the stand of seedlings and cases occur where frames are utterly worthless on that account.

The loss of plantable seedlings is, however, not the most important aspect of the disease. Frames with a relatively good stand of plants, i.e. with small or insignificant patches of disease, have a deceptive appearance of soundness, and it is the growers' experience that a large percentage—not infrequently 50 to 75 per cent.—of the plants succumb to *Botrytis* within a few weeks of being set out in the field. The gaps have to be filled in, sometimes more than once, before a reasonably good stand of plants is obtained. Over and above the extra labour involved is the consideration that each successive replanting postpones the date at which the crop will mature so that the advantages of the early market are to that extent lost.

The growers' experience, which was confirmed by Abdel-Salam in experimental work, is that the disease is accentuated by early sowing of the frames, e.g. at mid-October. Later sowing does not however meet the practical needs of the problem since the growers' aim is to have the seedlings as advanced as possible at planting-out time. Nor did Abdel-Salam's finding, that certain varieties, both of Cos and Cabbage types, are relatively resistant to the disease, solve the problem for these were slow in maturing and therefore inferior for the purpose in view.

A point with regard to the biology of the fungus which was clearly demonstrated by Abdel-Salam and which is significant to control measures relates to the mode of attack. The fungus may invade anywhere but by far the most frequent port of entry is via the cotyledons or the dead or dying lower leaves. In the crowded seedbed the lower leaves tend to become yellow and die, and at the time of transplanting may be represented merely by dead leaf bases. Such dead tissue is extremely liable to harbour the fungus and from there it readily proceeds to invade the stem. Realizing this Abdel-Salam attempted to disinfect seedlings at transplanting time by treating them for a short time with various fungicides. The majority of these treatments were harmful or useless. The only promising one was with a solution of Uspulun (or its variant, Nu-green), a proprietary mercurial compound. By immersing the seedlings for one hour in a 0·5 per cent. solution of the fungicide he was able to reduce the amount of disease, in the few experiments carried out, to about one-fifth of that shown in the controls. To judge from the figures quoted, however, one can state that Abdel-Salam was not dealing with seriously contaminated seedlings.

The continuation of the work over the period 1932-3 was taken up by Helali, whose results are incorporated in a part of a Ph.D. Thesis of London University (1933). He extended, among other things, certain lines indicated by the earlier work. In particular he examined more closely the effect of certain environmental conditions upon the incidence and severity of the disease, and produced a considerable body of evidence to show that low temperature, high air humidity and the disturbance of roots arising from transplanting all accentuate the disease. These results throw light upon an experience well known to growers, viz. that a batch of plants when set out in the open may be heavily attacked while a comparable lot still left in the frames may show little spread of disease. The transference from the drier and warmer soil of the frames to the wetter and colder ground of the fields, the exposure to rain or spring frosts, and the act of transplantation itself all contribute to intensify the disease, so that an attack which was almost dormant in the frames becomes definitely rampant.

The effect of manurial treatment of the field was also tested with results showing clearly that nitrogenous fertilizers increased the susceptibility to disease and that none of the treatments had any significant effect in the opposite direction. No control measure was to be developed along those lines.

Helali thoroughly tested the control measures suggested by Abdel-Salam. Over a two-year period he carried out an elaborate series of experiments with a variety of fungicides and varied modes of application, and with seedlings showing various degrees of contamination or infection. He confirmed Abdel-Salam that the only promising fungicide, as applied by immersing the seedlings, was Uspulun and claimed that, over the whole series of experiments, a treatment of one hour in a concentration of 0·5 per cent. of this fungicide reduced the mortality of planted-out seedlings to less than half that of the controls. This figure seemed to indicate the limits of the method, as the treatment bordered closely upon that which acted injuriously to the plants, and in particular to the roots. The check to growth was somewhat reduced when the modification of Uspulun known as Nu-green (= Uspulun with a certain admixture of an ammonium salt) was used and the latter was accepted as the best preparation then available.

The investigation was continued by the writer in the seasons 1934 and 1935, on a small scale in the former and much more elaborately in the latter year. These experiments will now be described.

Season 1934. The work of this year calls for a mere summary as its main value was to indicate the lines of research for the year following.

The seedlings available for planting, var. Trocadero, had been grown in the usual commercial manner in frames over winter, having been sown at mid-October of the year before. There was, as usual, considerable infestation, and the sample of plants was such that their survival on being transplanted to the open ground in March was in the neighbourhood of 20-30 per cent. That is, they were of a very inferior grade, but highly suitable for tests on fungicidal control.

Repetition of the tests with Uspulun confirmed the earlier work, viz. that there was appreciable control of the disease (percentage of survivors raised to 50-60), but that the method was restricted by the danger of damage to the plants. A similar conclusion was drawn from experiments with mercuric chloride (1 : 1,000). In this connection it is worthy of record that confirmation was obtained of the claim made by Raleigh,* that the fungicidal activity of mercuric chloride is considerably enhanced when enough potassium iodide is added to it just to redissolve the brick-red precipitate formed. In the writer's experiments, an immersion for five minutes in the mercuric chloride-potassium iodide solution was fungicidally as effective as one of twenty-five minutes in mercuric chloride alone, but unfortunately the damaging effect upon the plants ran in a like direction.

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* *Phytopath.*, 1933, 23, p. 28.

Tests with the non-mercurial dust "Brassisan" (the effective constituent of which is a chloronitrobenzene preparation) were included in the series since other work in progress had shown that this substance combined a high degree of fungicidal activity with remarkable safety to the plants concerned (*Brassica* types in relation to Club Root and Damping Off). The results were very promising from the beginning, e.g. in the first experiment a single dusting of the collars of seedlings, the roots being protected, gave the following numbers of survivors in batches of 100 plants:

Dusted	70, 76,	Av. 73·0
Controls	18, 28, 36, 28.	Av. 27·5

Two repetitions on a similar scale gave a like result. Incidentally it was shown that the dust was more effective when the treated plants were moist; hence the standard method of application was to spray the plants first with water, put on the dust and allow to dry. Presumably this treatment ensures better adhesion of the fungicide.

In none of the experiments with the dust so applied did the plants show any visible check to growth. It was only when plants were shaken up in a box with the fungicide so that the roots became liberally coated that a check appeared. Even so, the degree of disease control as measured by the number of surviving plants was high.

The safety of Brassisan to lettuce plants was further tested during the hot weather of 1934 by dusting with a bellows or by laying the dust thickly on the leaves of plants growing in the open. In no case was damage seen.

The provisional conclusion was therefore reached that so long as the roots are reasonably protected from contact with the dust, it could be used with perfect safety on lettuce.

Season 1934-35. The work of the preceding spring had shown that, with highly contaminated seedlings such as are obtained from frames in which the disease has made considerable headway, a large increase in the percentage of survivors could be obtained by a single dusting at the time of transplanting. The aim in 1934-35 was to repeat these experiments and also to see whether, by dusting the plants while they still grew in the frames, one could increase the number of plants carried by the growers through the winter and perhaps also their comparative freedom from contamination or infection.

Eight frames were sown (var. Trocadero) and given the treatments recorded in Table I. Otherwise the treatments were according to the usual commercial routine. The last column in the Table gives the number of plantable seedlings in the various frames at the time of transplanting (March 1935).

TABLE I.

Frame.	Date of Sowing.	Dusted.	No. of Seedlings in March, 1935.
A	15.10.34	5.12.34 and 24.1.35	675
B	,,	5.12.34	792
C	,,	None	210
D	,,	None	95
P	19.11.34	None	1,414
Q	,,	None	2,025
R	,,	5.12.34	6,810
S	,,	5.12.34 and 24.1.35	8,270

The dusting of 5.12.34 was carried out by first of all spraying the plants with water, then by vigorous blowing, first from one side then from the opposite, so that the underparts of the seedlings were reached. After treatment the soil and the plants showed a just visible coating of the grey powder. In a few places the dust was intentionally spilled, so that a layer which was clearly visible at transplanting time was left on the soil, the idea being to test the possible injurious effect of an overdose. On this point it may be said here that such a gross overdose did damage the leaves of young seedlings and is to be avoided. The possibility of a check to the growth of young seedlings will be considered later in this paper.

The second treatment was given in a simpler manner, viz. after the usual preliminary spraying with water, the frames were closed except to allow the entrance of the dusting nozzle and dust was then blown in until a light fog was produced. The lights were left on for some ten minutes until this cloud had settled, and were then taken off.

Frames A-D were sown earlier than most growers consider to be safe, but this was done to invite fungal trouble ; frames P-S, which were not available in time, were sown somewhat later than was desirable, but the seeding was made very heavy so as to favour the fungus. More disease was anticipated in the first range of frames as, in addition to being sown earlier, they had carried diseased crops for several years running, whereas the others were on fresh ground. Furthermore, at the date when the first dusting took place, viz. December 5th, disease patches were already showing in frames A-D but none in the others. These various considerations explain the much larger number of seedlings carried by the second range of frames than by the first.

From the point of view of the number of seedlings available for planting in the spring, the results of Table I may be summarized as follows:

Series A-D	Undusted	Av. 150
	Dusted	,, 730
Series P-S	Undusted	,, 1,720
	Dusted	,, 7,540

i.e. dusting increased the number of plants in each series some 4-5 times. The beneficial effect was obvious at a glance. The disease which had begun in frames A and B at the date of the first dusting was prevented from spreading, whereas in the control frames C and D it continued to spread and in the latter completely destroyed all plants except a few at the upper drier end. Frames P and Q showed numerous small bare areas whereas the comparable dusted ones (R and S) showed a dense unbroken stand of plants except for a small drip spot in the one and a similar small area in the other in which the fungicide had been intentionally spilt.

The figures given in Table I show that the first dusting was the significant one in increasing the stand of plants in the frames. Actually the double treatment gave more plants than the single in one series (S>R) but not in the other (A<B). No significance is attached to either of those differences. Nevertheless, clear evidence of the value of the second treatment was obtained when the plants were set out in the field, as will be pointed out below.

At the time of transplanting, sample lots (100 in each) were taken from frames P, R and S and examined for the presence of stem lesions. These, when present, almost invariably occurred at the base of withered lower leaves and cotyledons and it was not possible by macroscopic examination to distinguish between cases where the leaf base was withered and stem intact and others where invasion of the stem was just beginning. The figures below therefore refer to macroscopically visible stem lesions:

Frame P (undusted) .. 33, 29, 42, 25, 40 Av. 34·0 per cent.
,, R (once dusted) .. 1, 5, 3, 1, 4 ,, 3·0 ,, ,,
,, S (twice dusted) .. 3, 1, 2, 2, 3 ,, 2·2 ,, ,,

There was thus a striking difference in the quality of the seedlings from the point of view of freedom from invasion at the time of transplanting so that it was anticipated that the dusted seedlings would give a higher survival figure when planted out.

Seedlings were transplanted at intervals over March 1935, on five dates between the 4th and 29th, so as to experience a variety of weather conditions. The series of frames A-D, being the most advanced, were dealt with first. From about the middle of March extensive plantings were made from frames P (undusted) and S (twice dusted). Limitations of field space did not allow of similar treatment of frames Q (undusted) and R (once dusted), from which only a few samples were taken.

The seedlings were either transplanted without further treatment or were dusted (after preliminary spraying) a few hours beforehand so as to allow the fungicide to dry. In some experiments the seedlings to be planted out were first lifted, thoroughly mixed and then sorted into two comparable lots, one to be dusted and the other to serve as control. Such seedlings in order to be dusted had to be arranged in bunches and heeled in so as to protect the roots from the fungicide. This rather laborious method, which would be impossible on a practical scale, was considered to be advisable when the experimental seedlings were being taken from frames which showed a considerable number of disease patches. In such cases the sampling error might be high if the batches were taken from different areas of the frame. In general, however, and especially where there was a crowded mass of seedlings (e.g. frame S) the method adopted was to take out two spadefuls of soil from neighbouring places with as little disturbance as possible of the plants, one of these serving as control, the other being dusted without any further manipulation. The aim of this simpler method was to test whether it was practicable to dust the plants as they grew in the frame at the time of transplanting, i.e. when they formed a dense stand.

In the following account no distinction will be made between the experiments carried out by these two methods of dusting. In all cases where both methods were tested on similar batches of seedlings, equally good results were obtained. It was clear that a dense stand of seedlings could be dusted *in situ* provided the fungicide was vigorously blown amongst the foliage.

Altogether in the various plantings from the frames, some 7,000 seedlings were used, in batches which generally varied in the different experiments from 100 to 250. The seedlings in each batch were planted without selection. Any surplus sent out to the field to be planted was counted and discarded.

Records were taken at intervals of the number of survivors in the field and were continued in each case until no further appreciable losses occurred. The final records, given below, were taken on May 22nd, which was within a fortnight of the date at which many of the plants were well headed.

There being so few plants in frames C and D, the survivors were lifted, mixed and divided into two batches of 150 each.

The data relative to the behaviour of plants from frames A-D are assembled in Table II.

TABLE II.

Frame.	Treatment of Frame.	Plants.	Date of Transplanting.	Treatment at Transplanting.	Percentage Survivors on May 22nd.
C & D	None.	150	March 4th.	None.	9
,,	,,	150	,,	Dusted.	52
B	Once dusted.	100	,,	None.	17
,,	,,	167	,,	,,	16
,,	,,	100	,,	Dusted.	52
,,	,,	425	,,	,,	38
A	Twice dusted.	200	March 6th.	None.	57
,,	,,	175	,,	,,	51
,,	,,	200	,,	Dusted.	60
,,	,,	100	,,	,,	65

The results shown in the above Table may be summarized as follows:

(1) Seedlings from the early sown undusted frames (C and D), in addition to being few in number (Av. 150), show a negligible survival in the field. Nevertheless, a single dusting at the time of transplanting gave approximately a half-stand of plants.

(2) Seedlings from the once dusted frame (B), though much more numerous (790), were similar in quality to those from the undusted frames.

(3) Seedlings from the twice dusted frame (A) were comparable to the last in number (675) but gave a much higher survival when planted without further treatment, viz. 54 per cent. as against about 17 per cent. for those from frame B. The dusting of seedlings from frame A at the time of transplanting only slightly increased the percentage of survivors. Whether this was because the dusting was insufficiently done or whether the infestation of these particular lots was in the form of infection chiefly and not of contamination is unknown. It may be added that no adequate scrutiny was made of the plants from frames A-D with respect to the presence or absence of lesions.

The behaviour of sample plantings from frames P, Q, R and S is shown in Table III.

TABLE III.

Frame.	Treatment of Frame.	Plants.	Date of Transplanting.	Treatment at Transplanting.	Percentage Survivors.
P	None.	175	March 13th.	None.	59
S	Twice dusted.	175	,,	,,	94
P	None.	100	March 15th.	,,	53
,,	,,	,,	,,	,,	39
,,	,,	,,	,,	,,	42
,,	,,	,,	,,	Dusted.	77
,,	,,	,,	,,	,,	76
S	Twice dusted.	,,	,,	None.	79
,,	,,	120	,,	,,	78
,,	,,	,,	,,	,,	86
,,	,,	,,	,,	,,	83
,,	,,	,,	,,	,,	79
,,	,,	200	,,	Dusted.	89
P	None.	250	March 26th.	None.	47
Q	,,	,,	,,	,,	36
S	Twice dusted.	,,	,,	,,	69
,,	,,	,,	,,	,,	67
Q	None.	240	March 29th.	,,	10
,,	,,	,,	,,	Dusted.	44
R	Once dusted.	480	,,	None.	25

The figures for replicate experiments in the above Table give a general indication of the degree of uniformity shown. Though the sampling error may have varied considerably in the different tests, the following conclusions may safely be drawn:

(1) The survival of plants from frame P (undusted) is always considerably lower than that of plants from frame S (twice dusted), both being planted out without further treatment. This was in accordance with expectation based on the condition of the seedlings when examined for the presence of fungal lesions (v.p. 254).

(2) During the period of transplanting there is substantial evidence that the seedlings in the frames were deteriorating as regards ability to survive in the field, no doubt on account of progressively increasing overcrowding.

This result is clearly seen with the plants from frame S. These when planted out at the middle of the month gave a survival figure of approximately 80-90 per cent. whereas about twelve days later it had dropped to less than 70 per cent. The general tendency of the plantings from frames P and Q, which were equivalent, was likewise for the percentage of survivors to fall as time progressed. In the absence of deterioration in the frames, one would expect a higher percentage of survivors in the later plantings as the ground temperature is then higher with the result that the plants become more quickly established. A further consideration is that, as the frames were emptied from the lower ends upwards, successive plantings, other things being equal, should have been freer of disease, as attack in the frames is typically more pronounced in the moister lower part.

In this connection it is significant to note that the great majority of plants taken from frame S round about the middle of March had their cotyledons still intact, whereas these had almost invariably withered a fortnight later. Plants in the latter condition, unless some protection is given, are liable to be attacked through the dead cotyledons.

How far deterioration of the type described would occur under commercial conditions, would depend on the degree of overcrowding allowed, which would not be so intense as in the frames of this series, especially in those which had been dusted.

In the light of the results discussed under Table II it would have been interesting to make a sustained comparison of the plants from the once dusted (R) and twice dusted (S) frames. The interest of this, however, was not realized at the time. The sole planting from frame R was made late and showed distinctly poor survival in the lack of further treatment. Though a strict comparison cannot be made on the basis of available data, there is a strong suggestion that the increase in the survival figure produced by the second dusting, as pointed out above (page 252) for the series of frames A-D, is shown here also.

The most striking result achieved in these experiments is seen on comparing the record of frames C and D with that of frame S. Undoubtedly there are several points of difference in the respective treatments, but on the one hand the final result per frame was a crop of thirteen plants, whereas if all the seedlings from frame S had been set out at about the middle of March when their survival figure was about 85 per cent. at least, the crop would have approximated to 7,000 plants. It may be added that there was no noticeable difference between these two lots of plants in date of maturing.

A subsidiary experiment with seedlings in boxes gave results which strikingly confirmed the efficacy of the dusting treatment. This experiment will be briefly reviewed.

Thirty seed boxes were sown on November 30th and the plants brought on in a heated greenhouse. A few days after emergence of the seedlings, 16 of the boxes were lightly dusted, 4 heavily dusted, the remainder being untreated. When the plants were about one inch high all the boxes were distributed at random in two empty sloped frames and given the ordinary commercial attention henceforth. At the end of January four of the lightly dusted boxes were again similarly treated.

The idea of this experiment was to have a reserve of material in case high mortality should occur in the frames sown in the ordinary manner. This method of cultivation, however, proved to be unsatisfactory, because though the boxes were about six inches deep the plants began to wilt during February. They therefore had to be watered several times and to that extent the experiment did not conform with commercial practice. The result however was very clear, at the middle of March, when all the boxes were discarded. The twice dusted boxes gave a very good stand of plants, followed by the once heavily dusted lot. The boxes which had been lightly dusted once had abundant disease patches and the controls had no living plants at all.

An interesting and unexpected result was obtained with a series of plots of lettuce which had been drilled in the open ground (September 12th) so as to stand the winter outside. Altogether four (I-IV) such plots were available, each containing nine 10 ft. drills of seedlings. The rate of seeding was not accurately measured but was approximately uniform throughout. The seed—var. Trocadero—had had slightly different treatments in the various plots, the experiment being devised in another connection, but the details are irrelevant here as the seeding of each plot was uniform.

On account of the mild winter the plants of this series grew well and it was decided to test whether dusting would improve the yield of plants fully exposed to outside weather. Accordingly, the first and second rows of each plot were dusted three times (December 5th, December 23rd and January 24th), the remainder being untreated.

The numbers of living seedlings obtained from the various rows when transplanting took place in the first and second weeks of March are set out in Tables IV and V.

TABLE IV.

Row.	Treatment.	Plot I.	Plot II.	Plot III.	Plot IV.
1	Dusted.	99	71	58	87
2	,,	91	75	72	80
3	Undusted.	21	26	29	50
4	,,	19	36	32	56
5	,,	22	31	30	61
6	,,	26	21	28	57
7	,,	40	35	34	46
8	,,	37	49	53	87
9	,,	31	41	47	59

The average number of survivors per row is given in Table V.

TABLE V.

Treatment.	Plot I.	Plot II.	Plot III.	Plot IV.
Dusted	95	73	65	83
Undusted	28	34	36	59

The figures show quite clearly that, in spite of the wet weather to which the plants were exposed, a great improvement in yield of plants resulted from dusting. The beneficial effect was further and more strikingly illustrated when sample lots of these plants were set out and their survival figure determined. The relative data are assembled in Table VI.

TABLE VI.

Plot.	Treatment.	No. of Plants set out.	Percentage Survivors.
I ..	Dusted.	170	41
	Undusted.	60	3·3
II ..	Dusted.	146	61
	Undusted.	175	19
III ..	Dusted.	125	61
	Undusted.	100	29
IV ..	Dusted.	150	67
	Undusted.	150	30

Combining the results of Tables V and VI one obtains a rough estimate of the number of mature plants obtainable from each 10 ft. row of the original plots (Table VII).

TABLE VII.

	Plot I.	Plot II.	Plot III.	Plot IV.
Dusted ..	39	44·5	40	56
Undusted ..	1	6·5	10·5	18

Considering Plots I-IV as approximately equivalent, one obtains on summation the net result that the number of mature plants obtained from each unit of seed drill was increased five times by dusting.

The experiments with seedlings grown over winter in the open suggest a number of interesting points. The Trocadero variety is classed among the non-hardy varieties from the point of view of frost resistance, but the question may fairly be asked whether the general failure of Trocadero seedlings to last over winter in the open is due to frost in itself or to fungal invasion which is favoured by cold or wet conditions. The winter of 1934-35 was unusually mild but there were some spells of frost and there was a good deal of cold wet weather. The experiment is obviously worthy of repetition over a number of winters.

If it should be proved that, within fairly wide limits, the damage due to frost is largely indirect, viz. by diminishing resistance to the invasion of fungi and in particular of *Botrytis cinerea,* the further point might be made that the rather expensive method of overwintering in frames could be replaced by cultivation in the open ground. There is, however, a further complication which was very prominent in the plots of heading lettuce carried at the Slough Field Station during the spring and early summer of 1935. The disease due to *Marssonina* was favoured by the wet conditions prevailing and showed itself so early on the lettuce wintered in the open that a large proportion of this crop, though maturing a fortnight before those overwintered in the frames, was badly blemished and would scarcely have been marketable. On the other hand, the plants from the frames, though showing widely spread infection, were as a rule affected in only a few of the lowermost leaves and the great majority could have been marketed. Whether dusting could be performed in such a way as to control the *Marssonina* trouble is unknown, but it is safe to say that the success of the outdoor method of cultivation will depend in large measure on the solving of the *Marssonina* problem.

The question of possible damage to the plants by use of the fungicidal dust has already been raised. Experiments on this point, not merely with lettuce but with a large number of seed plants, are in progress and the results will be published in due course. The main points so far determined as regards lettuce are as follows:* The only case of severe damage was where the roots were liberally coated with the dust. No check to growth or other injury was noted when seedlings were dusted *in situ* a few hours before being pulled up and sent out, heaped up in a box, to the field, whereby the roots ran some risk of coming in contact with the dust. Plants dusted at the time of transplanting in the manner described in this paper invariably grew as well and headed out as soon as the undusted controls.

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* I am indebted for some of these particulars to Miss M. J. Smieton of the Slough Biological Field Station.

A slight check has, however, been seen, but not invariably, when young seedlings are dusted in frames or in the greenhouse. This takes the form of a slight mottling and puckering of the leaves on which the dust is laid and a delay in the development of the younger leaves, hence an appearance of dwarfing. This effect disappears within a few weeks. In the series of frames A-D, dealt with earlier in this paper, there was at the time of transplanting no difference in size between the plants of the undusted and twice dusted frames. In the series P-S there was a difference, but this was undoubtedly due to the extremely crowded state of the plants in the dusted frames. Even so, the plants from the latter grew to the same size in the field and matured as early as those from the parallel untreated frames. In the light of all the information available, which is fairly extensive, the risk of fungicidal damage may be dismissed as negligible.

At the moment there is little information as to the minimum effective dose or the number of profitable applications. As this dust is known, from other studies, to control the damping-off of lettuce (due to *Rhizoctonia Solani*) either when incorporated in the soil or dusted on the surface of the seedbed, it is probable that more than two treatments would be beneficial. The method of application is simple, whether the frames be traversed by the blower (which is probably the more effective method) or whether the dust be merely blown into the closed frame. The labour of monthly dustings from November to April is therefore not very great. Whether a preliminary wetting of the seedlings in the frame is advisable or not is uncertain, as the evidence in its favour is based on the results of treatments given in the open. Here again the labour involved in the additional operation is slight and the grower may avoid it altogether by choosing a still morning when the interiors of the frames are slightly damp from natural causes.

BOOK REVIEW

BRITISH STEM- AND LEAF-FUNGI (*COELOMYCETES*). By W. B. Grove. Volume I. Sphaeropsidales. Pp. xx. × 488 and 31 text figures. Cambridge University Press, 1935. 21s. net.

Mycologists (whether systematists or plant-pathologists) have often felt the need for a book that would enable them quickly to identify the numerous fungal forms that they find associated with leaf-spots or with lesions on stems and shoots. True there are Saccardo's *Sylloge* and Rabenhorst's *Kryptogamen—Flora*, but the latter is sadly out of date and the former is rarely available for reference outside science libraries. Many of these forms are included in the Fungi Imperfecti and their identification is not always easy even with the aid of the works mentioned.

The book under review will be particularly welcomed therefore by those advisory and research workers who wish to determine, in limited time, whether a particular fungus met with, under conditions suggesting parasitic habits, is one known or suspected of causing injury to cultivated plants.

The author's "British Rust Fungi. Uredinales" is well known to all who are interested in the microscopic fungi attacking plants, and the present book will be found a most useful companion to the former. It is to be followed by another, and the scope of the two volumes is described on the dust-cover of the book before us as "A detailed account of the British Fungi Imperfecti belonging to the Orders Sphaeropsidales and Melanconiales. The first volume includes all the Sphaeropsidales to the end of those which have colourless spores. The second volume will contain the Sphaeropsidales with coloured spores and the Melanconiales."

On the fly-leaf is quoted, " The world is so full of a number of things ", and in the preface facing this we read, " The Caelomycetous section of the 'Imperfecti Fungi' to be treated of in these volumes numbers more than 2,000 reputed British species, and many of these are well-known despoilers of our field-crops, our orchards, and our woods." After this one wonders how many agriculturists and fruit-growers would endorse Stevenson's words which complete the couplet.

The arrangement of the contents will make a special appeal to the busy worker. There is a concise key to the genera; each genus in turn is then treated more fully and the species are described under the host plants which are in alphabetical order, so that " running down " a particular species is attained with little trouble. Although ease of manipulation is a great feature of this book, the reviewer suggests that this would be enhanced if page references to the genera accompanied the key on pages xix. and xx., so that anyone starting with this key could turn at once to the description of the genus without having to refer to the index. Each species is briefly described but with sufficient details for identification, and with an indication of the type of lesion with which it is associated. The descriptions are accompanied by text figures showing the destinctive features of most of the genera. The book concludes with descriptions, in Latin, of twenty-five " new species ", an Index of Hosts and an Index of Binomial Names of the fungi.

H.W.

THE INCORPORATION OF DIRECT WITH PROTECTIVE INSECTICIDES AND FUNGICIDES

I. THE LABORATORY EVALUATION OF WATER-SOLUBLE WETTING AGENTS AS CONSTITUENTS OF COMBINED WASHES

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It has been shown repeatedly that the efficiency of sprays which exert a direct toxic action on insect or fungus is related to the ease with which the spray wets the insect or fungal surface. Thus Eyre and Salmon (7) found that to secure the full fungicidal effect of ammonium polysulphide solutions on the hop powdery mildew, the addition of an auxiliary substance to facilitate the wetting and penetration of the conidiophores by the spray was necessary, whilst Moore (20) reported that the efficiency of aqueous solutions of nicotine as contact insecticides may be increased 50 per cent. by the addition of soap. The addition of such water-soluble auxiliary agents has now become a widely used method of increasing the efficiency of direct insecticidal and fungicidal sprays and, in practice, these materials are known as " spreaders ".

To protective fungicides such as Bordeaux mixture or lime-sulphur, which are effective by virtue of the properties of an adherent spray deposit, the addition of spreaders has not yet become customary. Similarly with stomach poisons such as lead arsenate, which function through the formation of a foliage deposit poisonous to insects and of which the efficiency is dependent, in a manner analogous to the protective fungicides, upon the initial amount of deposit and its degree of retention, the addition of wetting agents is not generally practised. In this case, however, the use has been suggested of auxiliary agents which either serve as protective colloids to delay sedimentation and thereby ensure the application of a spray of uniform concentration, or which enhance, by adhesive or other action, the retentivity of the spray deposit (Woodman, 32). Although such materials are frequently called spreaders, it has been suggested by Martin (15) that useful practical purposes are served by referring to such materials as " stickers ", reserving the term " spreader " for substances which, like soap, assist in wetting the sprayed surface. Pending a fuller definition, which will be attempted below, it is convenient to refer to materials which function as spreaders in this limited sense as " wetting agents ".

It follows that if direct insecticides or fungicides are to be incorporated in combined washes with protective sprays, the simultaneous addition of a wetting agent is one method of securing the full effect of the direct constituent. The investigation of such combined washes thus involves the study of the influence of the addition of wetting agents to protective sprays. The effects of the wetting agents not only upon the biological activities of the protectant, but also upon the amount of initial spray deposit and its retention, have to be determined. Previous work upon these problems is confined mainly to that of Kearns, Marsh and Pearce (14) on combined washes based on lime-sulphur-lead arsenate as the protective material and that of Martin (16), who used Bordeaux mixture as the protective fungicide. It is possible that a limiting factor to the investigation of combined washes in which a water-soluble wetting agent is used has been the paucity of wetting agents suitable for use with lime-containing products such as lime-sulphur or Bordeaux mixture. Indeed the investigators mentioned were limited to the proprietary products Agral 1 and Lethalate Wetting Preparation, or to sulphite lye. Within the past two years, however, the number of wetting agents available has increased to such an extent that it has become necessary to undertake a detailed investigation of the wetting properties of these materials in order to select those most suitable for the extensive field work necessary to establish the merits of combined washes. It is proposed to limit the present paper to the description of a preliminary investigation of the possibilities of the laboratory evaluation of the efficiency of these products as spray materials, the sprays used for this work being simple aqueous solutions of the materials. Because the results have been obtained with simple solutions and with artificial surfaces, they should not be applied directly to practical conditions.

THE CLASSIFICATION OF WETTING AGENTS.

The great majority of the wetting agents so far introduced may be classified on the assumptions that wetting ability is a property due to surface activity and that surface activity is associated with the molecular structure of the material. The suitable combination of one or more groups polar to water with a bulky non-polar group is, in general, the molecular structure of a wetting agent. Thus the soft soaps consist of a long chain alkyl radicle terminated by the polar carboxyl group whilst the alkali resinates consist of complex ring structures upon which water solubility is conferred by carboxyl groups. The nature of the non-polar part of the molecule, whether long-chain or cyclic, affords a suitable basis for classification.

(1) *Long-chain derivatives :*

(a) *Fatty acid derivatives.*—As an example of the soaps, the most widely used wetting agent of this class, sodium oleate may be cited. The predominant active ingredient has the structure :

$$CH_3.(CH_2)_7.CH=CH.(CH_2)_7.COONa.$$

As the calcium derivatives are insoluble in water the soaps are useless as wetting agents with lime-containing protectants. The substitution of the carboxyl group by certain other polar groups gives rise to fatty acid derivatives which yield water-soluble calcium salts. In some cases these products employ the sulphonic acid group as the polar group, a bridging group being necessary to confer stability. Thus the active constituent of *Igepon A* may be regarded as the condensation product of oleic acid and isethionic acid, and of the structure :

$$CH_3(CH_2)_7.CH=CH(CH_2)_7.CO.O.CH_2.CH_2.SO_3Na.$$

This compound is somewhat unstable in alkali solution, a disadvantage not shown by *Igepon T*. The active constituent of the latter product is stated (Hilditch, 11) to be the condensation product of oleic acid and taurine :

$$CH_3(CH_2)_7.CH=CH(CH_2)_7.CO.NH^{*}.CH_2.CH_2.SO_3Na.$$

The combination of oleic acid and certain substituted ethylene-diamines gives rise to products such as Sapamine base (Hartmann and Kagi, 10). This material is itself insoluble in water but it combines with acids, for example hydrochloric acid, giving water-soluble wetting agents of the type :

$$CH_3(CH_2)_7.CH=CH(CH_2)_7.CO.NH.CH_2.CH_2.NH(C_2H_5)_2Cl.$$

It is noteworthy that in derivatives of this type the long-chain is in the basic part of the molecule, whereas in the soaps and Igepons it is in the acidic part. The compound above is precipicated by alkali, but conversion of Sapamine base to a quaternary ammonium salt, as in *Sapamine MS*, gives a product which functions as a wetting agent in the presence of free lime.

(b) *Fatty alcohol derivatives.*—The ability of the fatty alcohols to yield relatively stable sulphuric acid esters gives rise to an important group of soap substitutes. The representatives of this group suitable as commercial products are decided by the availability of the long-chain alcohol. By the hydrogenation of cocoanut fatty acids, the mixture of fatty alcohols known as "Lorol" is obtained. The appropriate sulphuric acid treatment of this material yields *Sulphonated Lorol* of which the predominant constituent is sodium lauryl (dodecyl) sulphate :

$$CH_3(CH_2)_{10}CH_2O.SO_3Na.$$

As cetyl alcohol is a naturally-occurring product, textile detergents containing, as the active ingredient, sodium cetyl sulphate :

$$CH_3(CH_2)_{14}CH_2.O.SO_3Na$$

are common representatives of this group.

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* Duncan (5) quotes a formula in which the hydrogen above asterisked is replaced by a methyl group.

As with the fatty acid derivatives, the amino-derivatives yield quaternary ammonium compounds, certain of which find use in the textile industry. The general formula of this type of compound is $\mathrm{R\text{-}N(R_1)_3Y}$, where R is a long-chain and R_1 a short-chain radicle, whilst Y is a salt forming group. (Stewart and Bunbury, 29.) Compounds of particular interest to the applied entomologist arise when insecticidal nitrogenous derivatives such as nicotine are substituted for ammonia in this type of product. Thus " Tinocine D " (Austin, Jary and Martin, 2) contain a long-chain alkyl nicotinium bromide and in the preliminary experiments recorded showed not only excellent wetting but good insecticidal properties.

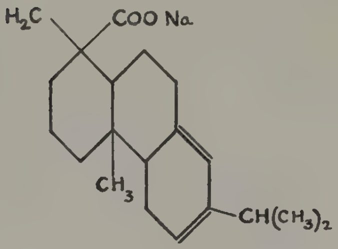
(c) *Fatty oil derivatives.*—The sulphonation of castor oil gives rise to Turkey Red Oil, an early soap substitute but which, like soap, yields insoluble calcium salts. The chemistry of the Turkey Red Oils is most complicated but it would appear that water-soluble properties are conferred by the introduction of the sulphuric acid ester group $-\mathrm{C}\vdots-\mathrm{O}-\mathrm{SO_3Na}$. Under drastic sulphonation conditions the true sulphonic acid group $-\mathrm{C}\vdots-\mathrm{SO_3H}$ may be introduced which confers greater solubility on the calcium salts, and a number of such highly-sulphonated glyceride oils are now on the market.

(2) *Cyclic derivatives :*

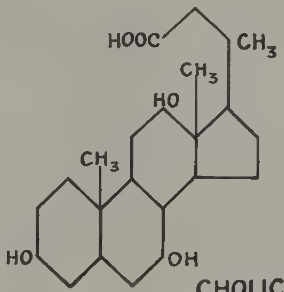
(a) *Aromatic derivatives.*—One of the simplest aromatic derivatives to be used as a spray spreader is sodium naphtholate, employed by Martin and Salmon (18) as a combined wetter-fungicide against hop powdery mildew. Both the $\alpha-$ and $\beta-$ naphtholates proved too phytocidal for general use, but, as is a general experience, the toxic properties are reduced by sulphonation. Naphthol and naphthalene sulphonic acids yield salts of moderate wetting power which is, however, stated to be markedly increased by the introduction of lower alkyl groups. These products are now widely used as textile wetting-out agents, the majority of which contain iso-propyl naphthalene sulphonates as the active ingredients. Certain naphthol derivatives of this type are also available.

(b) *Hydro-aromatic derivatives.*—The alkali resinates have long been used as emulsifiers and wetters in spray practice, but they yield insoluble calcium salts. The active constituents of the resinates are probably the abietates, to which the structure indicated below has been given. Sulphonation is said to increase the water-solubility of the calcium derivatives and a product stated to be a sulphonated abietene has been used as a wetting agent for spray residue removal (McLean and Weber, 19).

Bile salts may also be included in this group for the active constituents are derivatives of cholic acid, to which the structure indicated above has been assigned. Preparations of ox bile have been employed in France under the name "Adhesol" (Ballard, 3) as spray spreaders, and the purified product, sodium tauroglycocholate has long been available. As, on hydrolysis, this material yields cholic acid and taurine, it forms an interesting link, through *Igepon T*, between the cyclic and long-chain wetting agents.



ABIETATE



CHOLIC ACID

Examples of hydro-aromatic sulphonates suitable for use with calcium-containing sprays are to be found in the *petroleum-gamma sulphonates* (Martin, 17). The recently published work of Pilat and Szankowski (25) suggests that these products are the sulphonates of hydrocarbons of cyclic structure. The simpler of these hydrocarbons appear to be aromatic in character with no long side chains. It has been claimed that the introduction of lower alkyl groups into derivatives of this type yields improved wetting agents and the active constituents of *Agral N* are the sulphonates of cyclic hydrocarbons containing such aliphatic side chains.

The active constituents of *Sulphite Lye* (Martin, 15) also appear to be the sulphonates of complex cyclic derivatives for Sankey and Hibbert (27) and Howard (13) have shown the presence of ligno-sulphonates in this product.

(3) *Unclassified Products.*

In this group may be placed certain of the older spray spreaders of which the molecular structure has not been fully elucidated.

(a) *Proteins.*—Lime casein, the so-called "soluble caseins", which are probably the alkali salts of protein degradation products, and gelatine are the most widely used spray materials of this class.

(b) *Glucosides,* of which the most important members are the saponins.

THE LABORATORY EVALUATION OF WETTING AGENTS.

As the primary purpose of the present examination of wetting agents was the selection of materials for use in protective washes, attention was directed mainly to the effect of the wetting agent on the quantity of liquid initially retained on the sprayed surface. For this purpose a spray apparatus was devised for the application, under standard conditions, of known volumes of the aqueous solution of the wetting agent to various surfaces and for the estimation of the amount of spray retained. A description of this apparatus is given later, for it is convenient at this stage to discuss theoretical considerations leading to the conclusion that the results obtained by means of this apparatus may be correlated with certain physical properties of the spray.

In practice, the spray is invariably applied in droplet form and the formation of the spray deposit may be pictured as follows: In the extreme case of complete absence of wetting no spray deposit will be retained but, as the wetting properties of the spray increase, the amount of spray retained will increase with the volume applied until a certain maximum is reached. There appear to be at least two processes by which the maximum is attained. Firstly, in the case of poor wetting and spreading, droplets will impinge on an initial droplet retained, increasing the volume of that droplet until its weight overcomes the force retaining it to the surface, when it will run off, leaving the surface incompletely wetted (Types c and d, p. 282). Secondly, in the case of good wetting and spreading, as droplets impinge on an initial droplet, this droplet will coalesce with other droplets before its weight overcomes retaining forces. A film of liquid will thus be formed over the surface and excess of spray will drain off (Types a and b, p. 282).

As we are concerned with the action of materials added to improve the wetting properties of the spray, it may be assumed that the second of these processes will predominate, and it is evident that, in this process, the rapidity with which coalescence of the droplets occurs is dependent upon the area covered by individual droplets. The estimation of the surface area covered by droplets of standard volume might therefore furnish a measure of the amount of spray retained. This so-called "area of spread" has been employed by previous investigators as an index of wetting and spreading ability. Woodman (31) determined the area of spread of standard volumes of mixtures of ethyl alcohol and water on a waxed surface and showed that the area increased with decreasing surface tension. He also demonstrated that the smaller the volume of the drop the greater is the area covered per unit volume. O'Kane *et al.* (23) used the area of spread as a measure of the spreading or contact quality of a given liquid.

Theoretically the use of area of spread as a criterion of wetting ability is based upon the relationship

$$\gamma_{SV} = \gamma_{LV} \cos\theta + \gamma_{SL} \quad . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \quad (1)$$

which expresses the conditions for equilibrium when a drop of liquid of surface tension γ_{LV} rests upon a solid; γ_{SV} and γ_{SL} being the interfacial tensions solid-vapour and solid-liquid, and θ the angle of contact at the solid-liquid-vapour boundary, the angle being within the liquid. Equation (1) involves two other physical factors amenable to measurement, namely, the surface tension γ_{LV} and the contact angle θ, both of which have been used by earlier workers as indices of wetting ability.

Cooper and Nuttall (4) suggested on the basis of the relationship that, when $\gamma_{SV} > \gamma_{SL} + \gamma_{LV}$ . . . . (2), the drop tends to spread, and that reduction of the surface tension γ_{SL} will increase the spreading properties of the liquid. They verified this conclusion experimentally for surfaces coated with a thin layer of castor oil, and the determination of surface tension has since been adopted by Woodman (33) for the comparison of wetting agents. Cooper and Nuttall pointed out, however, that spreading and wetting phenomena are dependent on the three interfacial tensions involved and that it would be unwise to base conclusions on the estimation of one tension only. This view is supported by the work of Hamilton (8), whose results, summarized in Table I, show that surface tension is not a good criterion of the wetting properties and retentivity of sprays containing lead arsenate and spreaders such as saponin, casein, flour and glue.

TABLE I.

Spreader.	Surface tension dynes/cm.	Secs. to wet glass plate.	Wt. of lead arsenate retained.
Skim milk	60	5·0	0·88
Flour	60	6·5	0·47
Saponin	60	6·0	0·46
Casein	60	4·0	0·52
Glue	60	3·8	0·32
Glue	60	3·8	0·30

When equation (1) is expressed in the form :

$$\cos\theta = \frac{\gamma_{SV} - \gamma_{SL}}{\gamma_{LV}} \quad . . . . (3)$$

it will be seen that the contact angle is a function of the three interfacial tensions, and may therefore provide useful information concerning wetting agents. Thus, a drop of liquid exhibiting a contact angle of 180° would touch the surface at one point only, whereas if $\theta = 0°$ the droplet would form a film over the surface. In practice, values of θ intermediate between 0° and 180° would be obtained, the greater the angle the poorer would be the wetting and spreading properties of the liquid. The value of contact angle as a criterion of the wetting and spreading properties of sprays has been explored by Stellwaag (28), English (6) and by O'Kane *et al.* (21, 22, 24).

Contact angle and surface tension also appear as factors in the thermodynamic conception of " spreading coefficient ", a term introduced by Harkins,

$= \gamma_{LV}(\cos\theta - 1)$ . . . . . (4). The spreading coefficient was employed by Wilcoxon and Hartzell (30) as a measure of the spreading properties of insecticidal sprays.

EXPERIMENTAL METHODS.

From the foregoing discussion it will be seen that of the physical criteria of possible value for the assessment of wetting and spreading properties, spray retention, area of spread, contact angle and surface tension are involved. The technique used for the measurement of these properties is described below but, as all with the exception of surface tension, are dependent upon the nature of the solid interface, details are given first of the types of surface employed.

(1) *Surfaces used.*—In view of the variability of natural leaf surfaces, standardized artificial surfaces were found necessary for the detailed investigation of wetting and spreading properties. In selecting suitable surfaces, attention was paid to materials which would yield surfaces akin in chemical structure to the various plant and insect surfaces sprayed in actual practice. Thus the waxy surface of leaves, being of hydrocarbon structure, may be imitated by paraffin wax, providing a surface resistant to wetting by aqueous solutions. To simulate the cellulose surface, use was made of simple cellulose lacquers whilst shellac was used as a representative of an easily wetted insect surface.

Glass plates, 7 in. × 3 in., for spraying trials, and 7 in. × 1 in., for contact angle measurements, were dipped into molten paraffin wax (M.P. 60° C.) or into one of the following solutions: cellulose nitrate ($12{\cdot}0 \pm 2$ per cent. N., ash negligible), 2·5 per cent. in butyl acetate; cellulose acetate, 2·5 per cent. in acetone (three parts) and ethyl lactate (one part by volume), the latter acting as plasticizer preventing opaqueness in the cellulose acetate film; shellac, a saturated solution of orange shellac in absolute alcohol. After dipping, the glass plates and strips were allowed to drain and dry for eighteen hours before use.

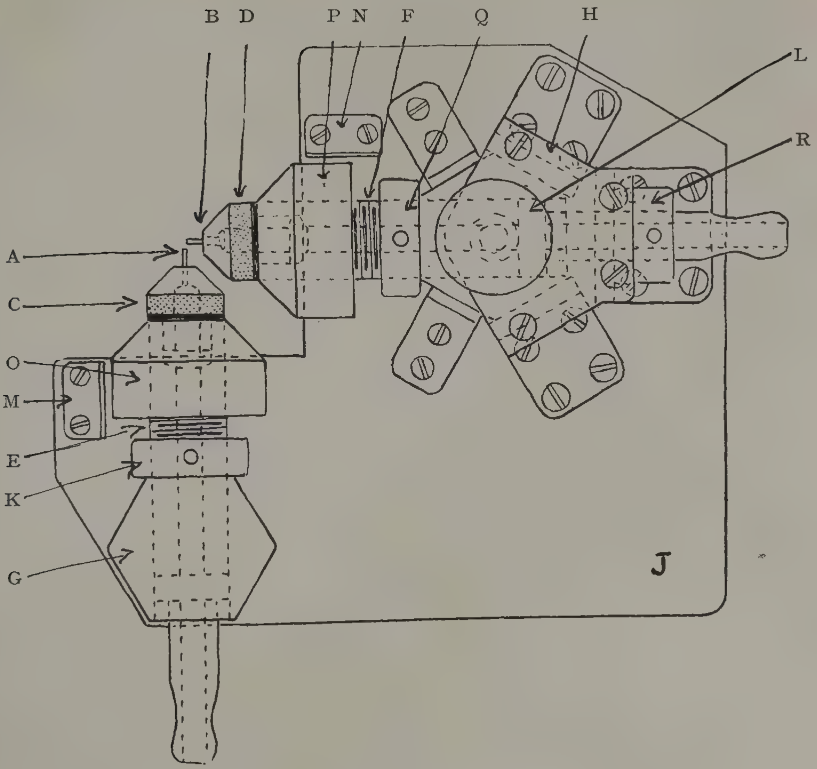
It is not suggested, at this stage, that these few surfaces are the most suitable of the many available nor that they satisfactorily represent all types of surface encountered in spray practice. Indeed experience has already indicated that shellac in particular is, on account of difficulty of standardization, of doubtful value.

(2) *Spray Apparatus.*—A simple small-scale apparatus was devised to study the behaviour of solutions when sprayed under standard conditions on standard surfaces. The apparatus shown in Plate I consisted essentially of an atomizer, a pressure regulator and a device for regulating the volume of spray applied.

The atomizer, shown in Fig. 1, consisted of two jets arranged at right angles, compressed air at a constant pressure of approximately 1·5 atmospheres passing through one jet atomizing the liquid delivered from the second jet by gravity

FIG. 1.

Atomizer.


B
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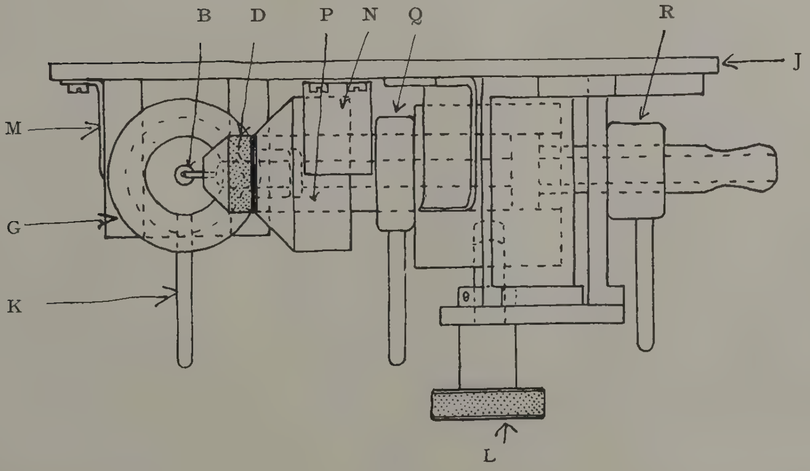
Elevation.


B
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Plan.

or by the suction effect of the air from the air jet. The jets A and B, constructed of stainless steel hypodermic syringe tubing, were mounted in screwed holders C and D, enabling the use of jets of various diameters. These holders were attached to screwed tubing, E and F, threaded into two bosses, G and H, fixed to the baseplate J. The vertical movement of the tube carrying jet A was fixed by a locking-nut K. Jet B had a similar adjustment for horizontal movement parallel to the baseplate but, in addition, provision was made for lateral movement controlling the alignment of the jets. This was actuated by the spring-loading of the tube F and controlled by the milled nut L, lock nuts being provided at Q and R. The relative position of the two jets could be reset by means of graduated scales, coarse adjustments being made by the side scales M, N and fine adjustments by graduations O, P on the jet holders. To obviate vibration, the whole unit was rigidly mounted at a fixed distance from the sprayed surface.

The volume of spray delivered to the surface under examination was controlled by means of a large screen 24 in. × 8 in. with a rectangular aperture 1·5 in. × 1 in. situated slightly to the side of the middle of the screen. The screen was attached to a pendulum so that the aperture swung directly across the centre of the spray cone. The rate of swing of the pendulum, a stout wooden rod 5 ft. long suspended by a steel knife edge working on a rigid slate bed, was controlled by allowing it to begin its swing at a fixed distance of displacement from the vertical and its amplitude maintained by a weight placed in a rigidly-fixed balance pan at the lower end of the pendulum. The surface to be sprayed was placed at a fixed distance from the spray jets and as over the short period of spraying the rate of swing of the pendulum is constant, the amount of spray reaching unit area of the surface sprayed is in simple proportion to the number of times the aperture of the screen passes over the surface.

The accuracy with which the amount of spray applied can be controlled by this apparatus is illustrated by the following figures obtained by weighing the amount of spray retained on a paraffin surface when a second fixed screen with an aperture 1 in. × 1 in. is placed behind the screen attached to the pendulum and close to the surface :

(a) Weight of liquid retained per sq. in. for thirty exposures (swings of pendulum) :

93, 96, 94 mg.

(b) Weight of liquid retained per sq. in. for twenty-two exposures :

116, 116, 116, 119 mg.

The amounts retained in trials (a) and (b), which were taken before run-off occurred, cannot be compared with each other as the adjustment of the spray nozzles, distance of nozzles from surface and the amplitude of the pendulum were not the same in the two series.

Although the volume of spray applied to the surface is determined by the number of exposures, it does not follow that the amount of spray retained by the surface can also be so measured. For this purpose either direct weighing of the surface before or after spraying, or the estimation of copper retained after spraying with standard copper sulphate-wetting agent solutions were used, though, as will be shown later, with the solutions examined, adequate information concerning retentivity was obtained directly from the volume of spray applied.

(3) *Area of Spread.*—The area of spread of drops of known volume of the solutions was determined on thin glass plates coated with films of cellulose nitrate, acetate or shellac, and placed on horizontal squared paper ruled in mm. squares. For the paraffin-waxed surface the squared paper was dipped into molten wax because, owing to the opaqueness of the film on glass plates, the squared paper beneath could not be seen.

The drops of liquid (0·06 ml.) were delivered from a small pipette made from capillary tubing and held with the tip just above the surface. The same pipette was employed throughout the investigation, for it was found necessary to use throughout drops of equal volume. O'Kane *et al.* (24) deduced theoretically that the area of spread would vary directly with volume to the power $\frac{2}{3}$: $A = KV^{2/3}$ . . . . . (5). Actual measurement, recorded in Table II, showed that direct proportionality was not obtained. K increases with volume of drop indicating a flattening effect of gravity upon the droplet :

TABLE II.

The Relation between Area of Spread and Volume of Droplet.

Volume c.mm.	Area of Spread sq. mm.	K (calc.).
10	16	3·45
50	50	3·69
100	85	3·95
150	117	4·14
200	141	4·12

Woodman (31) measured the area of spread of large volumes (1 ml., 5 ml. and 10 ml.) of liquid but as drops of this size are not usual in spray practice a drop (0·06 ml.) of the smallest volume giving areas of spread large enough for accurate measurement was used. That reproducible results can be obtained with small droplets of this size is illustrated by the following measurements, made on different dates, for the area of spread of distilled water on cellulose nitrate films : May 15th, 40, 44, 44 ; May 21st, 45, 41, 43 ; May 27th, 43, 42, 42 sq. mm.

To permit of the attainment of equilibrium the drop was left undisturbed for fifteen minutes before the area was estimated. The determinations recorded in Table III, which shows the increase with time of the area of spread of four drops of a 1 per cent. solution of sulphonated lorol on paraffin wax, indicate that this interval of time is sufficient for the attainment of the maximum area of spread.

TABLE III.

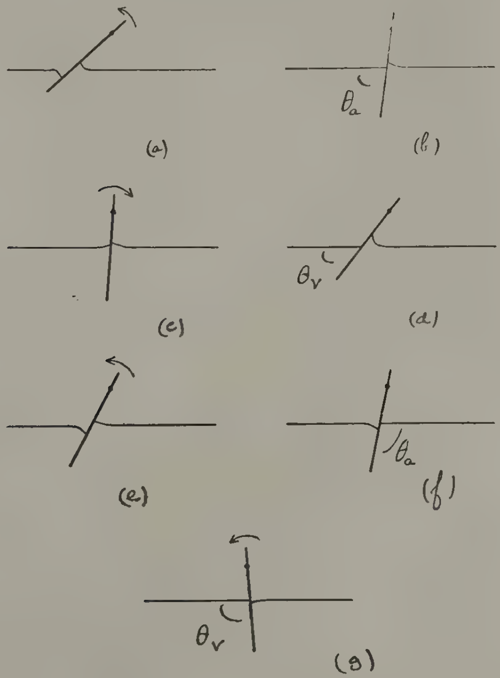
Time minutes.	Area of Spread (sq. mm.).			
	Drop I.	II.	III.	IV.
0·5	64	54	55	61
2	72	64	62	66
5	74	70	70	70
10	74	73	74	74
15	74	74	75	76

(4) *Contact Angles.*—The plate method employed by Adam and Jessop (1) was adopted for the measurement of contact angles, the apparatus used being a modification of that used by English (6). The modifications made were: firstly, provision for the smooth adjustment of the angle at which the plate is rotated in relation to the liquid surface by means of a slow-motion condenser dial of a type widely used in radio-receiving sets; secondly, the placing of the axis of rotation at a distance from the liquid surface which ensured that as the plate was rotated towards the vertical a fresh surface came into contact with the liquid and that the clips holding the plate did not enter the liquid, dirtying and distorting the liquid surface.

In the apparatus used, which is illustrated in Plate II, the glass plate treated with the required surface is held parallel to the pointer by two spring clips, one on either side of the axis which is rotated by means of the condenser dial. The liquid to be examined is placed in a trough with paraffin-waxed edges along which run paraffin-waxed strips which were swept over the liquid surface to clean it before each measurement. The slide is rotated anti-clockwise until it meets the liquid surface when it will be found that, on the left-hand side of the slide, the surface of the liquid will be depressed whilst, on the other side, it will be raised as in Fig. 2 (a). The surface is rotated further until the liquid on the left-hand side remains horizontal right up to the plate (Fig. 2 (b)). The contact angle is then read off on the protractor which is backed by a mirror to reduce parallax errors. This angle is termed the advancing contact angle, θa, as the liquid is, relatively speaking, advancing up the slide. The receding contact angle, θr, is determined by rotating the slide in a clockwise direction. The surface of the liquid now becomes raised on both sides of the slide (Fig. 2 (c)), and the slide is further rotated clockwise until the liquid surface on the left-hand side runs again horizontally right up to the slide (Fig. 2 (d)).

For the determination of advancing contact angles greater than 90° (as in Fig. 2 (e)), attention is directed to the surface of the liquid on the right-hand side of the slide, when the surface becomes level up to the slide the angle, as shown in Fig. 2 (f), is read. The receding angle is now determined by rotating the slide further in an anti-clockwise direction, when the level of the liquid on the left-hand side of the slide will continue to rise until it remains level up to the slide (Fig. 2 (g)). Once the slide has passed through 90° it is gradually withdrawn from the liquid with continued rotation, hence a receding angle is recorded.

FIG. 2.



(5) *Surface Tension.*—Surface tensions were measured by the drop volume method. The volume of the drop was determined by counting the number of drops formed from a known volume of the solution run from a calibrated Traube stalagmometer, and the surfaces tension calculated from the formula :

$$\gamma_{LV} = \frac{mg}{r} \times F \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (6)$$

where m is the weight of one drop calculated from the volume, the density of the solution being assumed to be 1, r the radius of the tip from which the drop falls and F is the correction depending on the volume of the drop and the radius of the tip of the stalagmometer (Harkins and Brown, 9). The tip of the stalagmometer must be ground flat and have a clear-cut circular rim.

EXPERIMENTAL RESULTS.

(1) *Analytical Data.*

The materials used in the preliminary survey of the applicability of the technique described above for the laboratory evaluation of wetting agents included the following :

Sodium Oleate.—A stock solution was prepared by dissolving 10 ml. of oleic acid (B.P.) in 990 ml. distilled water containing 3 gm. sodium hydroxide. The solutions for testing were obtained by the dilution of the freshly-prepared stock and the concentrations quoted are adjusted to the equivalent of a soap of 50 per cent. fatty acid content. Thus the solution described as 0·2 per cent. sodium oleate contained 0·1 per cent. oleic acid.

The purpose of this modified and arbitrary method of expressing concentration was to enable a rapid interpretation of the results to a practical basis, for the majority of soft soaps employed in spraying contain 40-50 per cent. fatty acid. For critical work it would seem preferable to adjust concentrations to a molar basis but as this procedure involves the assumption that the impurities, diluents or stabilizers present in the commercial products used are without effect upon the properties of the active constituents, it was considered unsuitable for the purposes of this survey. Attention has, however, been paid to the types of ingredients which accompany the active constituents of the products used. It should be pointed out that the fact that many of the wetting agents examined have been found to contain much material which cannot be regarded as active ingredients does not imply that these products are adulterated. Such material is frequently a simultaneous product of the manufacturing process and there may be economic and psychological reasons for not isolating the active constituents or marketing purer products.

Igepon T (Powder).—The solution after hydrolysis by 1 : 1 or 1 : 4 hydrochloric acid gave an ether-soluble fraction, an intermediate layer and an acid solution. After repeated hydrolysis and ether extraction the amount of intermediate layer became negligible and the total ether extract yielded 28 per cent. (of original powder) of fatty acid of neutralization value 190 and of 0·4 per cent. nitrogen content.

The powder contained 1·1 per cent. nitrogen and yielded 64·2 per cent. ash of 32·3 per cent. chloride content (on original powder) and 0·65 per cent. P_2O_5.

These figures indicate that the product contained about 40 per cent. of the taurine-oleate condensate product which would furnish 27·4 per cent. oleic acid (neutralization value 200 and containing no nitrogen) and 1·4 per cent. nitrogen, and which may be regarded as the active constituent. The concomitant material appears to be mainly sodium chloride (53 per cent.) and sodium phosphate.

Sulphonated Lorol.—On hydrolysis by 1 : 5 hydrochloric acid the powder yielded 29·5 per cent. fatty alcohol of acetyl saponification value 245. The acid solution after hydrolysis yielded about 6 per cent. sulphate (expressed as sulphur on original powder). These figures indicate a content of 45-47 per cent. sodium "lorol" sulphate.

As the powder gave only 30 per cent. ash and the total sulphur content was 12·2 per cent. it may be concluded that the concomitant material is organic in character but its precise nature was not determined. On reference to the manufacturers they have permitted the statement that the diluent is the sodium salt of a sulphonated hydrogenated cyclic hydrocarbon.

C72.—Hydrolysis by 1 : 5 hydrochloric acid yielded 27 per cent. ether-soluble alcohol of acetyl saponification value 190. As the acid layer yielded 3·5 per cent. sulphur, the paste may be estimated to contain some 38 per cent. of sodium cetyl sulphate (requiring 27 per cent. alcohol of acetyl saponification value 197·5 and 3·5 per cent. sulphur). Ether extracts also furnished an intermediate layer resistant to hydrolysis, soluble in water but readily salted out. In view of its high emulsifying properties, this material may be regarded as a true sulphonic acid and as contributing to the wetting and spreading properties of the paste. Its amount, not greater than 5 per cent., was too small to permit of its quantitative separation and examination.

The loss on drying at 100° C. (slight charring) was 58 per cent., and the paste yielded 3·5 per cent. total sulphur and a faint borate reaction with turmeric paper. It may therefore be concluded that the major ingredient of the paste, other than sodium cetyl sulphate and the sulphonate, is water.

Agral 2.—Because of the difficulties attending the elucidation by analytical methods, of the structure of the alkylated naphthalene sulphonic acids, no attempt was made to determine the precise character of the active constituents of wetting-agents of this type. Attention was directed solely to the examination of concomitant material unlikely to possess wetting and spreading properties. For this purpose two methods were adopted, ash content and percentage insoluble in alcohol, both of which, if high, indicate the presence of inorganic material. Both methods fail, however, to yield accurate estimates for, on ignition, reduction of the sulphate (formed from the sulphonate) by carbon (formed from the organic derivatives present) with the formation of sulphide gives low results for sulphate and sulphonate content. Further, because of the probable hydrotropic action of the sulphonates, the alcohol solubility figure is likely to be high and the percentage insoluble in alcohol correspondingly lower than the true content of inorganic material insoluble in alcohol. The assumption has been made that the alcohol-soluble fraction may be regarded as active constituents, and the possibility that these constituents are mixtures of compounds of different activities is ignored.

Agral 2 yielded 32 per cent. of ash and 12 per cent. sulphur (by combustion with cupric nitrate), whereas 25 per cent. proved insoluble in absolute alcohol. It may thus be assumed that the content of active ingredients, stated by the manufacturers to be alkylated naphthalene sulphonates, is of the order of 75 per cent.

C75.—This product, stated by the manufacturers to be a naphthol sulphonic acid derivative, yielded 51·9 per cent. of ash, contained about 18 per cent. total sulphur and gave 48 per cent. alcohol-insoluble residue. The alcohol-insoluble material showed but slight capillary activity and was mainly sodium sulphate. The content of active constituents therefore appears to be of the order of 50 per cent.

Agral N.—The alcohol-insoluble fraction, which amounted to 17 per cent. of this product, showed strong capillary activity and it appears probable that the actual content of active constituents, stated by the manufacturers to be alkylated sulphonates of cyclic hydrocarbons, approaches 100 per cent.

Crude Calcium Gamma-sulphonates.—This product was prepared from an acid sludge from transformer oil refinement by the method described previously (Martin, 17). Analysis showed a content of 10·7 per cent. sulphur and 11·8 per cent. calcium oxide. The material proved almost insoluble in cold absolute alcohol, but soluble to the extent of 88 per cent. in 83 per cent. by volume ethyl alcohol, the insoluble fraction being almost devoid of wetting and spreading properties. As the ash amounted only to 23 per cent. it may be concluded that the product is of low content (about 10 per cent.) of inactive material.

Sodium Resinate.—An alcoholic solution of commercial colophony, to which caustic soda had been added in amount determined by titration of an aliquot with phenolphthalein as indicator, was evaporated down and taken up in water to give a solution containing 10 per cent. of the original resin. The clear solution was decanted from insoluble matter and used as stock solution for the preparation of the test solutions. The stock solution (10 per cent. resin) yielded 5·56 gm. per 100 cc. resin acids yielding insoluble barium salts, 0·76 gm. per 100 cc. resin acids yielding soluble barium salts and 0·34 gm. per 100 cc. neutral ether-soluble material.

Saponin.—Hopkin and Williams' " Pure ".

TABLE IV.

	Conc. %	Mg. spray retained/sq. in.				Area of spread sq. mm.				Cos θ a.				Cos θ r.				Surface tension.
		Par	Cn	Ca	Sh.	Par	Cn	Ca	Sh.	Par	Cn	Ca	Sh.	Par	Cn	Ca	Sh.	dynes/cm.
Long-chain derivatives: Sodium Oleate ≡Soap of 50% f.a.	1·0	96d	13a	—	—	66	138	—	—	·62	·82	—	—	·68	1·0	—	—	30·3
	0·4	89d	13a	11a	11a	95	250	256	—	·72	·90	·94	·96	·76	1·0	1·0	1·0	30·8
	0·1	57d	20a	21a	30d	89	337	276	184	·89	·97	> ·97	> ·97	·91	1·0	1·0	1·0	31·6
	0·05	153d	132d	101d	93d	42	49	73	53	·10	·42	·77	·48	·16	·63	·82	·69	71·8
	0·00	178d	236d	157d	170d	32	43	53	47	−·31	·17	·49	·26	−·23	—	·71	·60	73·0
Igepon T ..	1·0	62d	19a	—	—	82	305	—	—	·70	·95	—	—	·88	1·0	—	—	29·7
	0·2	74d	22a	13	19a	82	218	250	—	·67	·92	> ·97	> ·97	·85	1·0	1·0	1·0	35·9
	0·05	97d	31b	21	27b	80	156	244	—	·48	·82	> ·97	·92	·62	1·0	1·0	1·0	44·9
	0·025	106d	42b	31	42b	75	149	228	170	·50	·78	·96	·86	·66	1·0	1·0	1·0	50·6
	0·010	147d	94c	67	78c	67	91	213	174	·34	·65	·90	·77	·52	·99	·99	·99	66·3
	0·000	219d	198d	161	173d	—	43	67	45	−·31	·16	·57	·28	−·23	·81	·79	·65	73·0
Sulphonated Lorol	1·0	126d	20a	—	—	78	410	—	—	·72	·85	—	—	·84	1·0	—	—	29·3
	0·5	139d	24a	13a	26a	78	285	332	324	·62	·82	> ·97	·84	·81	1·0	1·0	1·0	30·8
	0·2	152d	30a	—	—	69	189	—	—	·48	·75	—	—	·70	1·0	—	—	37·8
	0·1	161d	36a	24a	30a	59	92	315	274	·41	·69	·96	·58	·57	1·0	1·0	1·0	47·5
	0·05	175d	45b	34a	38a	55	75	290	83	·22	·60	·96	·49	·44	1·0	1·0	1·0	57·4
	0·025	184d	73b	—	53a	47	61	162	179	·09	·54	—	·43	·25	·99	—	·99	64·7
	0·010	194d	116d	95b	—	—	—	—	—	−0·10	·45	·82	—	−·2	·98	·98	—	—
	0·000	222d	181d	156d	—	—	—	—	—	—	—	—	—	—	—	—	—	—
C72	1·0	99	23	—	—	67	84	—	—	·60	·84	—	—	·68	1·0	—	—	35·2
	0·5	96	25	17	25	66	84	308	89	·59	·68	> ·97	·62	·68	1·0	1·0	1·0	37·8
	0·2	96	27	—	—	65	84	—	—	·55	·65	—	—	·61	1·0	—	—	41·0
	0·1	110	31	24	29	57	79	151	86	·43	·62	·91	·49	·51	·99	·99	·99	45·5
	0·05	124	36	28	36	60	72	133	80	·33	·58	·87	·44	·41	·98	·98	·98	52·6
	0·025	128	55	43	51	57	64	160	70	·23	·56	·80	·41	·26	·98	·98	·98	64·8
	0·000	195	193	150	219	—	—	—	—	—	—	—	—	—	—	—	—	—

TABLE IV.—*Continued.*

	Conc. %	Mg. spray retained/sq. in.				Area of spread sq. mm.				Cos θ a.				Cos θ r.				Surface tension
		Par	Cn	Ca	Sh.	Par	Cn	Ca	Sh.	Par	Cn	Ca	Sh.	Par	Cn	Ca	Sh.	dynes/c.m.
Cyclic derivatives :																		
Agral 2	1·0	116	23b	—	—	62	199	—	—	·66	·88	—	—	·79	·99	—	—	31·8
	0·5	141	27b	23a	24	57	129	265	146	·62	·82	> ·97	·86	·72	·99	·99	·99	34·6
	0·2	174	35b	—	—	45	61	—	—	·28	·62	—	—	·50	·99	—	—	46·3
	0·1	190	50d	34c	49	42	50	90	64	·20	·50	·80	·57	·35	·99	·99	·99	54·6
	0·05	202	95d	79d	78	37	44	74	57	·09	·42	·75	·51	·18	·92	·97	·95	64·4
	0·025	201	142d	106d	129	—	—	—	—	—·13	·31	·67	·42	·00	·87	·93	·87	70·2
	0·000	218	195d	157d	197	—	—	—	—	—·31	·14	·59	·33	—·23	·77	·81	·68	—
C75	1·0	70d	19a	—	—	75	424	—	—	·76	·98	—	—	·85	1·0	—	—	27·4
	0·5	76d	19a	—	—	79	419	—	—	·72	·97	—	—	·84	1·0	—	—	28·1
	0·2	109d	24b	20	24	64	141	272	128	·69	·84	·93	·81	·76	·99	·99	·99	34·4
	0·1	128d	32c	25	32	54	86	150	83	·52	·72	·87	·64	·59	·99	·99	·99	43·3
	0·05	159d	48	37	62	44	63	114	69	·26	·59	·80	·51	·31	·99	·99	·99	55·2
	0·00	223	188	165	209	—	—	—	—	—	—	—	—	—	—	—	—	—
Sodium Resinate ..	1·0	132d	30a	—	—	61	132	—	—	·57	·85	—	—	·77	1·0	—	—	32·5
	0·5	126d	31a	30a	30a	50	62	76	61	·46	·44	·72	·50	·62	1·0	1·0	1·0	37·6
	0·2	175d	61d	45b	46b	34	41	—	—	·07	·28	·54	·39	·13	·99	1·0	1·0	53·2
	0·1	206d	87d	—	—	33	41	52	49	—·21	·21	—	—	—·10	·99	—	—	61·6
	0·0	216d	192d	161d	202d	—	—	—	—	—	—	—	—	—	—	—	—	—
Crude Calcium γ — sulphonates	1·0	66d	25b	—	—	61	112	—	—	·55	·83	—	—	1·0	1·0	—	—	34·3
	0·5	78d	30b	—	—	61	104	—	—	·56	·78	—	—	1·0	1·0	—	—	35·7
	0·2	110d	33b	28b	31b	56	81	201	97	·54	·77	·92	·80	·99	·99	1·0	1·0	40·4
	0·05	211d	43b	41b	50b	45	59	127	72	·43	·69	·86	·52	·81	·98	·98	·98	58·4
	0·02	248d	69c	76c	70c	40	54	101	63	·11	·49	·76	·45	·26	·97	·98	·98	71·6
	0·01	234d	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	0·00	235d	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

	Conc. %	Mg. spray retained/sq. in.				Area of spread sq. mm.				Cos θ a.				Cos θ r.				Surface tension.
		Par	Cn	Ca	Sh.	Par	Cn	Ca	Sh.	Par	Cn	Ca	Sh.	Par	Cn	Ca	Sh.	dynes/cm.
Agral N	1·0	141d	22a	—	—	61	127	—	—	·58	·87	—	—	·83	1·0	—	—	32·4
	0·5	150d	27a	19a	33a	61	113	349	116	·55	·84	> ·97	·91	·79	1·0	1·0	1·0	36·2
	0·2	172d	31a	—	—	53	75	—	—	·38	·66	—	—	·64	1·0	—	—	46·7
	0·1	187d	47a	39a	39a	46	66	133	75	·22	·52	·78	·57	·34	·99	·99	·99	55·4
	0·05	205d	82b	76b	49b	41	53	116	64	·16	·48	·74	·50	·38	·99	·99	·99	65·6
	0·025	194d	151b	127c	80c	—	—	—	—	–·16	·34	·69	·81	·17	·94	·99	·99	—
	0·000	192d	—	153d	—	—	42	59	44	–·31	·25	·60	—	–·23	·77	—	—	—
Sulphite lye (60° Tw.) ..	1·0	312d	38c	—	—	36	48	—	—	·16	·35	—	—	·23	1·0	—	—	67·3
	0·5	325d	46c	116	76	33	43	55	46	–·18	·28	·63	·39	·06	·99	·99	·98	70·7
	0·2	243d	56c	—	—	—	—	—	—	–·26	·23	—	—	–·05	·99	—	—	71·7
	0·1	197d	111c	133	162	—	—	—	—	–·31	·16	·57	·28	–·20	·93	·81	·75	—
	0·0	200d	187d	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Miscellaneous Spray Auxiliaries :																		
Gelatine	0·5	221d	41b	48b	—	33	47	—	—	–·16	·22	·67	—	·27	1·0	1·0	—	66·5
	0·2	253d	41b	—	—	—	—	—	—	–·25	·21	·63	·44	·18	1·0	1·0	1·0	66·1
	0·1	303d	40b	51b	39	—	—	—	—	–·31	·20	·64	—	–·09	1·0	1·0	—	68·3
	0·05	298d	39b	55b	40	—	—	—	—	–·31	·18	·67	·45	–·12	·99	·99	·99	70·4
	0·025	330d	47b	65b	48	—	—	—	—	–·29	·20	·48	·40	–·17	·99	·99	·99	—
	0·000	246d	195d	180	197	—	—	—	—	—	—	—	—	—	—	—	—	—
Saponin	1·0	43c	36c	—	—	44	51	—	—	·07	·43	—	—	1·0	1·0	—	—	57·0
	0·5	48c	—	—	—	44	—	—	—	·02	—	—	—	1·0	—	—	—	60·6
	0·2	46c	36c	30a	39b	41	50	108	54	·00	·44	·81	·66	1·0	1·0	1·0	1·0	64·3
	0·05	68c	40c	31b	44b	38	47	100	53	–·16	·28	·77	·54	·99	1·0	1·0	1·0	68·8
	0·01	426d	98c	132d	87b	33	46	80	53	–·32	·21	·72	·43	> ·97	·99	·99	·99	73·3
	0·005	415d	131d	152d	120d	—	—	—	—	–·29	·16	·63	·35	·23	·98	·97	·98	—
	0·000	205d	194d	161d	213d	—	—	—	—	–·31	—	—	—	—	—	—	—	—

Gelatine.—Coignet's Gold Leaf.

Sulphite Lye.—The syrup of 60° Tw. supplied by Olive & Partington Ltd. was used, concentration being stated on a weight basis. Thus 1 per cent. approximates to the 6 pints syrup per 100 gallons spray recommended in practice. The syrup yielded 58 per cent. total solids, 8·7 per cent. ash and, as the lime content was less than 0·1 per cent., it may be concluded that the material is of soda base.

(2) *Physical Data.*

For the examination of the wetting and spreading properties of the above products, distilled water solutions were prepared, the initial concentration tested, in all cases except gelatine, being 1 per cent. Subsequent determinations were made with solutions of lower dilutions until concentrations were reached which yielded results akin to those for water alone. For ease of presentation, the results are tabulated in Table IV, in which each value is the mean of two or more concordant determinations. Before proceeding with the discussion of these data, it is necessary to explain certain specific points, mainly in connection with the spray retention figures recorded in Table IV, which it is convenient to separate from the general description of experimental technique.

(3) *Notes Concerning Physical Data.*

Retention Figures.—The amount of spray retained on the surface was first determined, by direct weighing before and after spraying, and it was found that the amount was, with the surfaces and solutions under examination, dependent solely upon the volume of spray applied. Thus Table V. shows the amounts of distilled water retained by the various surfaces and the amounts of various solutions retained by the paraffin-waxed surface under similar experimental conditions.

TABLE V.

Mg. spray retained when the volume applied is less than that required to give run-off or drain-off.

Surface.	Wt. distilled water retained. mg. sq. inch.	Wetting agent 0·1 per cent. solution.	Wt. retained on paraffin surface. mg. sq. inch.
Paraffin Wax ..	61 ; 60 ; 64	Sulph. Lorol	61 ; 62
Cellulose nitrate	62 ; 60 ; 64	Igepon T	63 ; 61
Cellulose acetate	64 ; 62 ; 62	Sapamine MS.	63 ; 61
Shellac ..	62 ; 62 ; 59	Nekal A.E.M.	64 ; 61
		Sodium-tauro-glycocholate	60 ; 62
		Agral N	61 ; 62
		Saponin	60 ; 63

These results indicate that the whole of the spray applied to the surface is retained until either run-off or drain-off occurs, a hypothesis which was confirmed by the comparison of the weight of spray retained by a paraffin-wax surface (72, 76 and 72 mg. per sq. inch) and by absorbent filter paper (72, 78 and 74 mg. per sq. inch). With the materials under examination and under the conditions of spray application it is evident that the spray retention figures can be obtained directly by determining the volume of spray applied which, in turn, is determined by multiplying the volume applied per sq. inch per exposure by the number of exposures.

Further, it was found that the maximum figure for spray retention is obtained by the visual observation of the number of exposures required to produce run-off or drain-off. In Table VI comparisons are made of the weight or volume of spray retained, the number of exposures and the visual observation of run-off. It will be seen that the relation between the number of exposures and weight of spray retained in linear until run-off occurs.

TABLE VI.

Retention at Run-off.

1% $CuSO_4 \cdot 5H_2O$ on cellulose nitrate surface.

No. of exposures.	Mg. Cu on surface.	Run-off.
50	0·24	—
100	0·49	—
140	0·68	Commencing.
145	0·48	—

Distilled water on paraffin-wax surface(A)

No. of exposures.	Mg. water retained.	
	By weight.	Theory.
10	33	33·5
20	65	67·0
30	97	100·5
40	133	134·0
50	167	167·5

Distilled water on paraffin-wax surface (B).

No. of exposures.	Wt. of spray retained (mg.).
8	40
16	82
24	122
32	160
34	171 Run-off at 37 exposures.
40	161 134
48	137
56	131 87
64	119
72	113 96
80	113
88	143
100	137

That the maximum figure for retentivity coincides with the point of run-off is one interesting justification of the recommendation, based on practical experience, that in the application of protectants such as Bordeaux mixture care should be taken to avoid drip. The figures quoted in Table VI show that irregular and lower figures are obtained when the volume of spray applied exceeds that required for run-off and confirm the previous observations of Hockenyos and Irwin (12) on the deposition of Bordeaux mixture.

The procedure for the estimation of the retention figure is therefore reduced to the observation of the number of exposures required to give run-off or drain-off. The volume of spray applied per exposure was estimated before and after each run of observations by the determination of the weight of water retained on a paraffin surface after thirty exposures. As is to be expected it was found that there were slight variations in the number of exposures required for run-off. Calculation of the retention figure gives, however, concordant results, illustrated by Table VII, in the case of water on paraffin-wax surfaces, which, of the surfaces used, gave the most variable results. The apparent independence of the retention figures at run-off and the number of exposures suggests that time factors, such as the interval between exposures, have little influence. As no detailed work was carried out upon the importance of time factors, it should be mentioned that throughout the experimental work recorded in the present paper, the amplitude and frequency of the pendulum were maintained uniform throughout to give exposures each of 0·175 seconds at intervals of approximately 1·235 seconds.

TABLE VII.

Calculation of Retention figure from exposure number and weight figures.

Date.	Wt. water retained after thirty exposures.	No. of exposures to run-off.	Retention figure. mg. per sq. inch.
22/3	93, 96, 94	67, 77, 65	219
26/3	84, 83	63, 68, 77	192
29/3	86, 84, 83	70, 67, 71	195
3/4	81, 83	79, 81, 80	218
9/4	86, 88	69, 71, 72	205
10/4	72, 72	97, 93, 94	201
16/4	70, 72	100, 90, 93	223
30/4	75, 75	94, 93, 95	235
7/5	69, 70	84, 89, 86	200
15/5	111	58, 59	216

In addition to observations of the volume required for run-off, the nature of the spray deposit was noted. These observations are recorded in Table IV by the suffix a, b, c or d to the retention figure ; (a) indicates that the surface was perfectly wetted by the volume of spray required for drain-off, whereas (b) indicates a nearly perfect wetting which could be converted to a perfect wetting by continuing the spray application beyond the point of run-off ; (c) indicates that the liquid could not be made to give a perfectly wetted surface by continued application but a streaky spray pattern is formed, and (d) indicates that run-off occurs without leaving a trail of liquid behind. It should be pointed out that in cases where drain-off occurs the retention figures refer to the initial amount of spray retained. The actual amount retained after draining although possibly influenced by this figure will be determined more by the viscosity of the spray and by the rapidity of evaporation. In this respect our use of the term retention differs from that used by Woodman (31) who determined, on perfectly wetted glass surfaces the amount of spray retained after a definite time, and whose retention figures are therefore mainly determined by the viscosity of the sprays used.

Contact Angles.—The advancing and receding contact angles are expressed in Table IV as the cosines. For the determination of angles approaching a cosine of 1 the apparatus was unsuitable, and an indirect method was used. If on withdrawal of the surfaced plate from the liquid the film of solution is maintained unbroken it is evident that the receding angle is zero. If the film slowly receded from the surface, the contact angle was considered to be of cosine 0·99, whereas a rapid retreat indicated a contact angle of cosine 0·98 or less. Angles of cosine less than 0·97 were directly determined.

DISCUSSION.

If, for the time being, the term "activity" be used in referring to the ability of the solute to cause a departure of the figures for retention, area of spread, contact angle and surface tension of the solution from those of water, it is evident that the wetting agents and spray "spreaders" examined differ widely in activity. On the other hand there is a general correlation, with any one auxiliary material, between concentration and the various phases of activity for, when plotted on squared paper, a smooth curve is obtained in the majority of cases. The only exceptions are the products C72, which shows fluctuations in area of spread on paraffin-wax and cellulose nitrate surfaces, and Sulphonated Lorol which exhibits irregular areas of spread on the shellac surface.

In the case of the cellulose nitrate, acetate and shellac surfaces activity increases with concentration of solute. Thus the retention figure falls, slowly in the case of sulphite lye, and extremely rapidly with Igepon T, to a minimum which appears to differ according to the adjunct used. The same order of substances giving the lowest retention figures, i.e. sodium oleate, Igepon T, C75, Sulphonated Lorol and C72, is followed upon all these surfaces. Upon the paraffin-wax surface, however, in addition to curves falling more or less rapidly to a minimum retention figure, certain of the materials, e.g. Agral N, gamma-sulphonates, gelatine and saponin exhibit a maximum before falling to a minimum figure. This inflexion is shown most markedly by saponin, which rises to the highest and ultimately falls to the lowest of the retention figures recorded. Sodium oleate and, possibly, sulphite lye, show the reverse phenomenon, falling at first, and then rising to retention figures which, in the case of sulphite lye, exceed that of water alone.

The curves for area of spread and the contact angles exhibit greater regularity and, with all materials except sodium oleate and possibly C75, which have maxima on the paraffin-wax surface, show a slow or rapid increase of activity with concentration. Because of the different slopes of the curves and of the absence of data concerning the maximum activity required of a spray, it is impossible to select definite concentrations for comparison of the various materials on a scientific basis. It was therefore decided to select concentrations which would cover those likely to be used or actually used in spray practice, namely 0·2 and 0·05 per cent. in the case of saponin, sodium oleate and the textile wetting agents, 0·5 and 0·1 per cent. for gelatine and 1 per cent. in the case of sulphite lye. The figures obtained at these concentrations, interpolated where necessary, are indicated in Fig. 3. In this figure the results obtained with cellulose nitrate, acetate and shellac surfaces are placed together because of their general similarity whilst the various materials are spaced upon columns of equal height, water being at the base and the most active material at the head of the column, the position of the symbols being proportional to the activity of the solutions of the selected concentrations.

The columns are arranged arbitrarily around surface tension in order to stress certain similarities. Receding contact angle figures for cellulose nitrate, acetate and shellac surfaces corresponding to those for paraffin-wax surfaces, have been omitted for, at the concentration of 0·05 per cent., all the wetting agents examined revealed angles of cosine 1 with the exception of Agral N, Agral 2 and sodium oleate on cellulose nitrate, Agral 2 and sodium oleate on cellulose acetate and shellac. It is noteworthy that these materials occupy relatively low positions in the columns (12, 13 and 14) for retention.

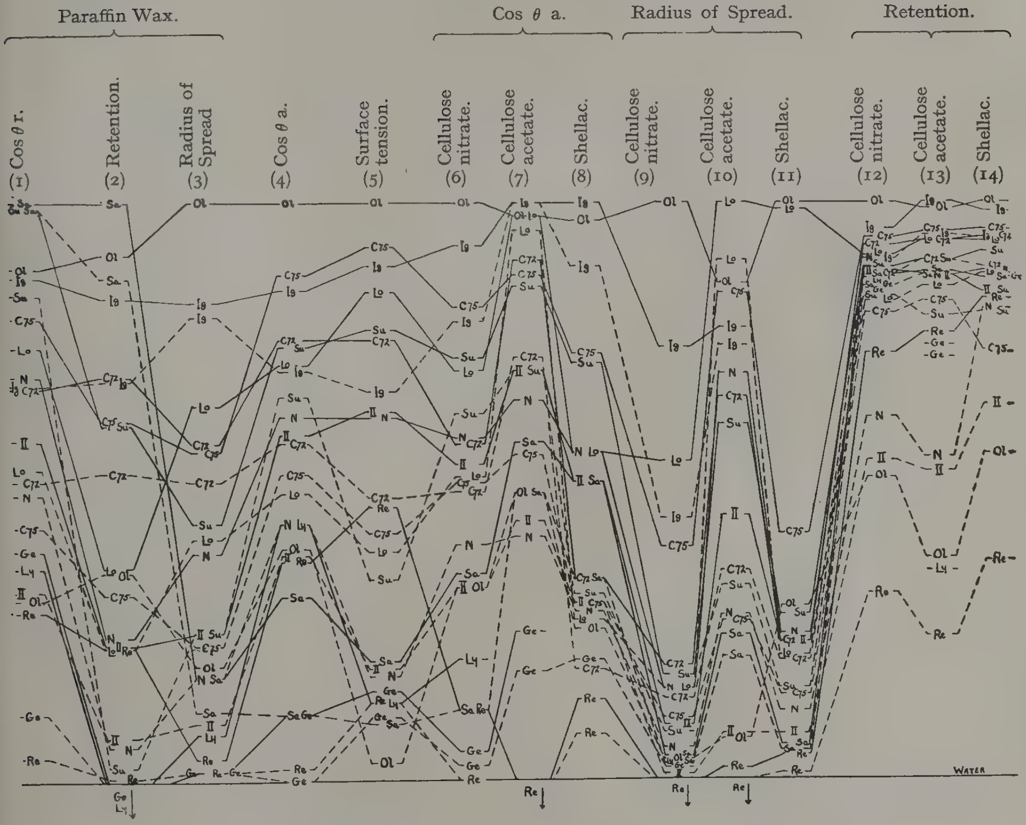
Inspection of Fig. 3 reveals that in each column the same general order of materials is maintained but for a few striking exceptions, e.g. saponin, Sulphonated Lorol. Such differences in order of activity result in the intersection of the connecting lines between the columns, often at wide angles. Further, it is apparent from the large differences in relative activity shown by the wide fluctuations up and down the columns of the connecting lines for any one material, that the various items determined supply divergent quantitative estimates of activity. To facilitate discussion it is therefore convenient to analyse Fig.3 by dealing in turn with the correlations between the various items determined.

(a) *Retention and Receding Contact Angle.*

The close correlation between the retention figures and the receding contact angles for cellulose nitrate, acetate and shellac surfaces has already been mentioned. Evidence of a similar correlation is shown in the case of the paraffin-wax surface (columns 1 and 2, Fig. 3), and it is possible to explain, on

FIG. 3.

Comparative Activities of Wetting Agents.



Ol= Sodium Oleate
Ig= Igepon T
Lo= Sulphonated Lorol
II= Agral 2
Re= Resinate
Su= Calcium gamma-sulphonates
N= Agral N
Sa= Saponin
Ge= Gelatine
Ly= Sulphite Lye

——=0·2%
— — =0·05%
——=0·5%——=0·1%
——=1%

this basis, the high retention activity of saponin by the low receding contact angle of its solution. Such an explanation fails, however, to account for the wide divergence of materials such as the gamma-sulphonates (Su) nor is the correlation well shown between the various concentrations used of saponin, gelatine and sulphite lye.

It has been suggested in the preliminary discussion of the factors determining the retention figure (p. 265) that two processes may be involved, one resulting in drain-off, the second in run-off. The first process occurs when the surface is easily wetted and it is to be expected that solutions of good wetting properties will furnish low retention figures. Such solutions are indicated in Table IV by the suffices (a) and (b). An almost perfect correlation is shown between such solutions and the receding contact angle. In every case upon surfaces other than paraffin-wax, solutions of receding angles of cosine of 0·99 or 1·0 give either the (a) or (b) type of wetting. On paraffin-wax no solution used gave perfect wetting though saponin (Sa) and the gamma-sulphonates (Su) yield low receding contact angles. It should be pointed out that the low receding contact angles were determined by the indirect method in which wetting properties are used as the criterion. But the indirect method involves the liquid at rest and the attainment of equilibrium and it is, in this sense, a static method. The retention figure, on the other hand, is determined by actual spraying and the correlation is an indication that the contact angle method provides an adequate measure of the wetting properties of the spray.

Throughout the above paragraph the expression "wetting properties" has been used, and it is now possible to define this term more closely. The wetting properties of a liquid are determined by the ability of that liquid to form a stable liquid-solid interface when excess of the liquid is drained from the surface. A water-soluble wetter is accordingly a material which yields aqueous solutions of wetting properties greater than water. From the experimental data recorded, it may be concluded that the determination of the receding contact angle provides a quantitative measure of wetting properties. This conclusion affords a satisfactory theoretical basis for the widely used test of horticultural "spreaders" which consists of immersing a wax-coated card in the spray solution and observing that the card remains wetted on withdrawal. As the wetting properties of a particular liquid have been shown to be influenced by the nature of the surface wetted, it is suggested that, if the results of this test are to be applied to practice, the actual leaf surface and spray should be substituted for the waxed card and wetter solution.

(b) *Retention and Area of Spread.*

If the exceptional case of saponin (Sa) on the paraffin-wax surface be omitted, there is a close correlation between the retention figure and the area of spread expressed as radii of spread in columns (2) and (3), (9), (10), (11), and (12), (13), (14) of Fig. 3. The greatest discordance is shown by Sulphonated Lorol (Lo), which exhibits a high radius of spread well shown on cellulose acetate (column 10), but evident also on paraffin wax. The utility of area of spread as a measure of spray retention suggested in the introductory section is thus supported by the experimental results, though it is clear, in view of the exceptions pointed out, that other factors in addition to area of spread determine the amount of spray initially retained. Of these factors wetting properties appear of importance for the kinetic energy of the spray droplet may impart to it an area of spread which is determined by the receding contact angle.

The term " spreader " has been loosely applied (see e.g. Woodman, 33) in horticultural practice to all types of auxiliary spray materials. It is, nevertheless, convenient to retain the term " spreading properties " to describe the ability of a liquid to form a liquid-solid or liquid-liquid interface by extension solely by surface activity over the surface of a solid or non-miscible liquid. The determination of area of spread, though static, would appear fundamentally sound as a method for comparing the spreading properties of liquids. It may, however, be necessary to distinguish between the phenomenon of spreading on a plane surface such as was used in the above-described experiments, and the property which enables certain liquids to creep into the trachea of insects or to penetrate dense felt-like masses of fungal hyphae. It is not suggested at this stage that a solution of good spreading properties as defined above will of necessity also be of good penetrating properties.

Applying these definitions to the experimental results it may be said that sodium oleate is an efficient wetter and spreader on all surfaces examined, that saponin is an excellent wetter but poor spreader on paraffin surfaces, that Sulphonated Lorol is a better spreader than Igepon T on the cellulose acetate surface but inferior on the paraffin surface.

(c) *Area of Spread and Advancing Contact Angle.*

The correlation between these two properties is of particular interest for O'Kane *et al.* (23, 24) have suggested that the contact angle provides a method of appraisal of so-called " wetting ability ". They considered that the determination of the area of spread of droplets of similar volume gives a direct measure of " contact qualities " but, as difficulties were met in the estimation of area of spread, they substituted contact angle measurements. It would appear that the property which they term " wetting ", " spreading ", " contact " and " extension " is that described as " spreading " above, and that the angle of contact which they employed was, in all cases, the advancing angle.

From Fig. 3 it will be seen that, for any one surface, the radius of spread and advancing contact angle arrange the wetting agents examined in the same general order of activity. Thus the high position of Sulphonated Lorol (Lo) in radius of spread upon cellulose acetate (column 10) is paralleled by a high position in advancing contact angle (column 7). It is noteworthy that the materials (Ol, Ig, Lo, C72) which are relatively low in order of activity as judged by advancing contact angle, yet are of relatively high spreading properties and which give, for example, almost horizontal connecting lines between columns (3) and (4), are of long-chain structure. To illustrate this feature the areas of spread interpolated for solutions of the same advancing contact angle, namely of cosine 0·6, for paraffin wax and cellulose nitrate, are given in Table VIII. The substances showing the greatest areas of spread are of long-chain structure.

TABLE VIII.

Area of Spread of Solutions of $\cos\theta\ a = 0{\cdot}60$.

Paraffin.		Cellulose nitrate.	
	sq. mm.		sq. mm.
Igepon T	82	Igepon T	80
Sulphonated Lorol	77	C72	76
Sodium oleate	65-75	Sulphonated Lorol	75
C72	67	Agral N	72
C75	58	Sodium oleate	65-70
Agral 2	56	C75	63
		Agral 2	59
		Gamma-sulphonates	55

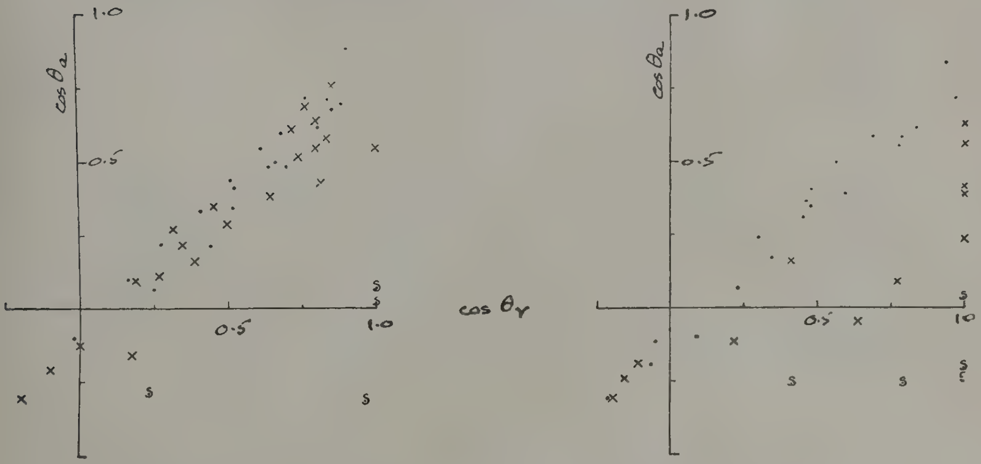
On the theoretical basis used by O'Kane, Westgate, Glover and Lowry (23), equal areas of spread should be given by drops of equal volume and of similar contact angles. The results summarized in Table VIII do not fully conform to this theory, and it is apparent that area of spread, and hence spreading properties, is determined by factors in addition to those which govern contact angle. If, as appears to be the case, the additional factors are determined by molecular structure, it would seem that they are associated with adsorption phenomena.

(d) *The Advancing and Receding Contact Angles.*

That there is a difference between the advancing and receding contact angles greater than can be accounted for by experimental error was recognized by Rayleigh (26), but the phenomenon was almost ignored until Adam and Jessop (1) put forward the suggestion that the difference was due to frictional forces. By accepting as the equilibrium contact angle that of which the cosine is the arithmetic mean of the cosines of the advancing and receding angles, they eliminated the effects of this friction.

In the use of contact angle for entomological problems, Stellwaag (28), English (6) and O'Kane *et al.* (21, 24) used only the advancing contact angle, whilst Wilcoxon and Hartzell (30) employed the equilibrium contact angle as defined by Adam and Jessop. The significance of the results of these investigators is therefore dependent on the correlation between the advancing and receding contact angles. To illustrate this correlation in the case of paraffin-wax surfaces, the cosines of the advancing and receding contact angles obtained with the various solutions are plotted in Fig. 4 (a). Points represented by dots are of solutions of the long-chain derivatives, sodium oleate, Sulphonated Lorol, Igepon T and C72, and the crosses are of the cyclic wetting agents, Agral N, Agral 2, C75 and calcium gamma-sulphonates. If the results with saponin solutions, indicated by S_1 are excepted a high correlation coefficient is obtained, and the close approximation to a straight line would indicate a constant difference between the cosines of the two contact angles.

FIG. 4.
Relationship between cos θ a and cos θ r.



(a)

(b)

It would therefore seem to be of little importance whether the advancing or receding equilibrium contact angles are used to arrange the wetting agents in order of efficiency. If, however, criteria such as Harkin's spreading coefficient involving the quantity (cos $\theta - 1$) are used, the choice of contact angle becomes of importance. In view of the value of the receding angle as a measure of wetting ability, and, further, of the exceptional behaviour of saponin, it would seem of value to regard the advancing and receding angles as separate entities.

During the preliminary work upon the determination of contact angles it was observed that a time factor is involved, for if the plate be rapidly rotated to the advancing contact angle and allowed to rest the meniscus slowly creeps.

The slowly determined advancing angle is lower than that rapidly determined whereas the rapidly determined receding angle is lower than that slowly determined. This phenomenon is illustrated in Fig. 4 (b) in which the dots represent the results obtained with solutions of Sapamine MS, sodium oleate and Igepon T, whilst the crosses are the results given by solutions of sodium tauroglycocholate, Nekal and Agral N. It is perhaps significant that the wetting agents which show most markedly the effects of this time factor are of cyclic structure.

This difference affords the second indication of the possibility that factors determined by type of molecular structure affect the relationships between spray behaviour and physical properties. It would therefore appear probable that the examination of a number of wetting agents of similar molecular structure would furnish more definite evidence upon the adequacy of the various physical properties under investigation as criteria of the performance of wetting agents of that type under spray conditions.

ACKNOWLEDGMENTS.

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SUMMARY.

1. For the purpose ot selecting for field use the most promising of the numerous wetting-out agents, detergents and other products now available as " spreaders " in combined washes, a laboratory method for the determination of spray retention is described.

2. This method has been applied to the evaluation of the wetting and spreading properties of aqueous solutions of these materials and to the examination of the suitability of the physical characteristics of area of spread and contact angles upon standard surfaces and of surface tension as criteria for the evaluation of wetting and spreading properties.

3. The detergents, wetting-out agents and other materials of possible value as " spreaders " are classified on a structural basis and analytical data of those selected for the preliminary trials are recorded.

4. Wetting properties are defined by the ability of the liquid to form a persistent liquid-solid interface when excess of liquid is drained from the surface.

A high correlation is shown between the wetting properties and the receding contact angle of the spray. Perfect wetting results when this angle is zero.

5. Spreading properties are defined by the ability of the liquid to form a liquid-solid interface solely by surface activity over the plane surface of a solid, and are, for the time being, distinguished from penetrating properties which determine the creep of the liquid through a porous solid. Spreading properties may therefore be assessed by the estimation of the area of spread.

6. The maximum amount of spray initially retained upon the standard plane surface held rigidly at right angles to the direction of the spray, is determined by both the wetting and spreading properties of the spray for that surface and shows a marked decrease with increase of wetting and spreading ability.

7. The area of spread is determined by the advancing contact angle, but in solutions of similar advancing contact angle examined, is greater with spreaders of long-chain structure.

8. The receding and advancing contact angles of the solutions examined exhibit a high degree of correlation, with the exception of saponin solutions. There is accordingly justification for the use of the equilibrium contact angle but, for reasons given, it is considered advantageous to regard the advancing and receding angles as distinct entities.

9. The various physical properties examined, namely, spray retention, area of spread, contact angles and surface tension, all arrange the materials tested in the same rough general order of activity. Except in the case of the correlations mentioned above, the determination of any one characteristic is insufficient to give a general assessment of wetting and spreading properties. The results obtained suggest, however, that with materials of similar molecular structure, it may be possible to deduce generalizations upon behaviour as a spray spreader and wetter from a limited number of laboratory-determined characteristics.

REFERENCES.

(1) *Adam, N. K.*, and *Jessop, G.* (1925). J. Chem. Soc., 1863.

(2) *Austin, M. D.*, *Jary, S. G.*, and *Martin, H.* (1932). Hort. Educ. Assoc. Yearbook, **1**, 85.

(3) *Ballard, P.* (1933). "La Mouillabilité des Bouillies." Reprinted from Revue de Viticulture.

(4) *Cooper, W. F.*, and *Nuttall, W. H.* (1915). J. Agric. Sci., **7**, 219.

(5) *Duncan, R. A.* (1934). Ind. Eng. Chem., **26**, 24.

(6) *English, L. L.* (1928). Illinois Division Nat. Hist. Survey, **17**, Art. 5.

(7) *Eyre, J. V.*, and *Salmon, E. S.* (1916). J. Agric. Sci., **7**, 473.

(8) *Hamilton, C. C.* (1930). J. Econ. Entom., **23**, 238.

(9) *Harkins, W. D.*, and *Brown, F. E.* (1919). J. Amer. Chem. Soc., **41**, 499.

(10) *Hartmann, M.*, and *Kagi, H.* (1928). Z. angew. Chem., **41**, 127.

(11) *Hilditch, T. P.* (1933). Rep. Prog. Appl. Chem., **18**, 406.

(12) *Hockenyos, G. L.*, and *Irwin, G. R.* (1932). Phytopath., **22**, 857.

(13) *Howard, G. C.* (1934). Ind. Eng. Chem., **26**, 614.

(14) *Kearns, H. G. H.*, *Marsh, R. W.*, and *Pearce, T. J. P.* (1932). Ann. Rept. Long Ashton Res. Sta., 66.

(15) *Martin, H.* (1932). Hort. Educ. Assoc. Yearbook, **1**, 76.

(16) *Martin, H.* (1933). Ann. Appl. Biol., **20**, 342.

(17) *Martin, H.* (1933). J. Soc. Chem. Ind., **52**, 429T.

(18) *Martin, H.*, and *Salmon, E. S.* (1934). J. Agric. Sci., **24**, 469.

(19) *McLean, H. C.*, and *Weber, A. L.* (1931). J. Econ. Entom., **24**, 1255.

(20) *Moore, W.* (1918). J. Econ. Entom., **11**, 443.

(21) *O'Kane, W. C.*, *Westgate, W. A.*, *Glover, L. C.*, and *Lowry, P. R.* (1930 a). New Hampshire Agric. Exp. Sta., Tech. Bull. 39.

(22) *O'Kane, W. C.*, and *Conklin, J. G.* (1930 b). Ibid., Tech. Bull. 40.

(23) *O'Kane, W. C.*, *Westgate, W. A.*, *Glover, L. C.*, and *Lowry, P. R.* (1931). Ibid., Tech. Bull. 46.

(24) *O'Kane, W. C.*, *Westgate, W. R.*, and *Glover, L. C.* (1932). Ibid., Tech. Bull. 51.

(25) *Pilat, S.*, and *Szankowski, W.* (1935). Petroleum, **31**, No. 10, 1.

(26) *Rayleigh, Lord* (1890). Phil. Mag., **30**, 397.

(27) *Sankey, C. A.*, and *Hibbert, H.* (1931). Canadian J. Res., **5**, 1.

(28) *Stellwaag, F.* (1924). Z. angew. Entom., **10**, 163.

(29) *Stewart, A.*, and *Bunbury, H. M.* (1935). "Colloidal Electrolytes." Reprinted from Trans. Faraday Soc., 208.

(30) *Wilcoxon, F.*, and *Hartzell, A.* (1931). Contr. Boyce Thompson Inst., **3**, 1.

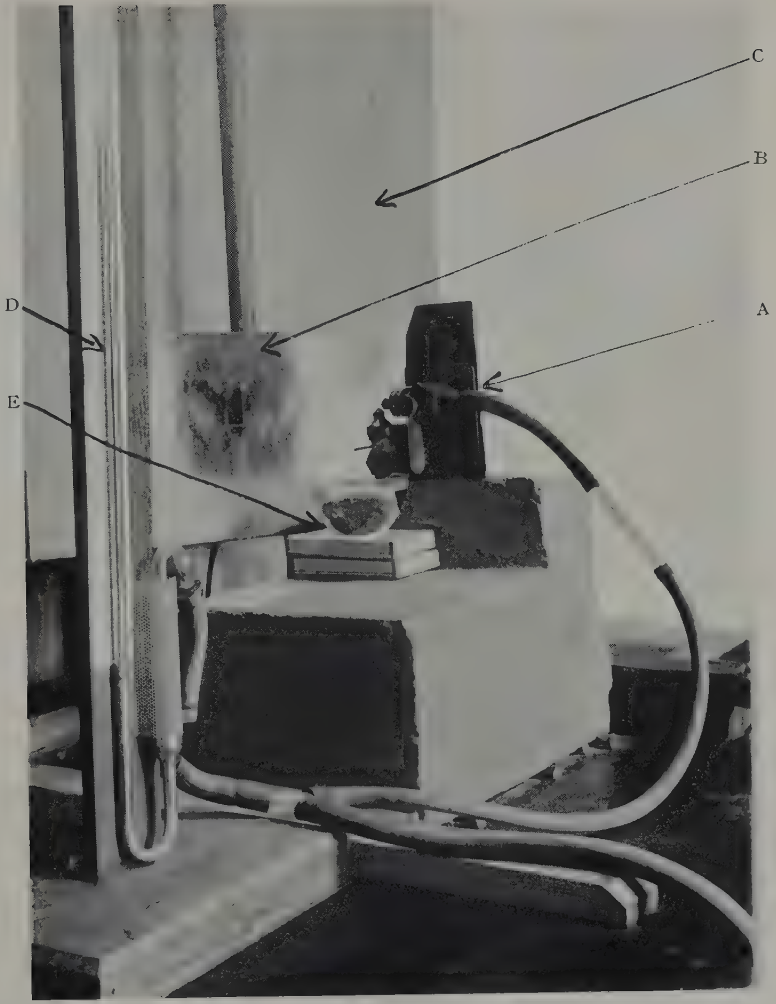
(31) *Woodman, R. M.* (1924). Journ. Pom. and Hort. Sci., **4**, 38.

(32) *Woodman, R. M.* (1924). Ibid., **4**, 78.

(33) *Woodman, R. M.* (1930). J. Soc. Chem. Ind., **49**, 93T.

(*Received* 26/8/35.)

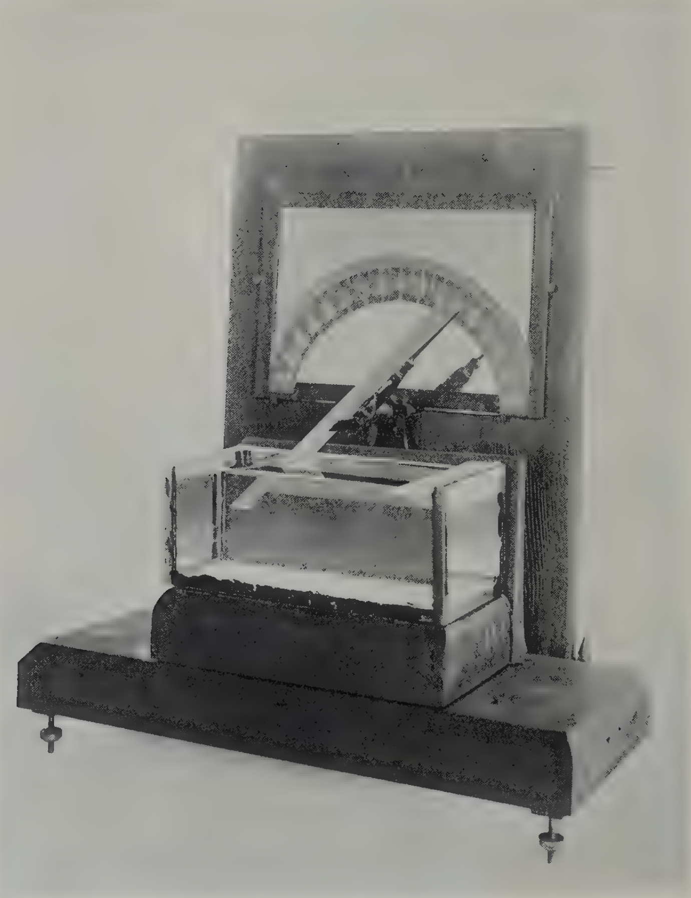
PLATE I.



Spray Apparatus.

A = Atomizer.
B = Pendulum and screen.
C = Support for sprayed surface.
D = Manometer.
E = Spray liquid.

PLATE II.



Contact Angle Apparatus.

APPLE ROOTSTOCK STUDIES

EFFECT OF LAYERED STOCKS UPON THE VIGOUR AND CROPPING OF CERTAIN SCIONS

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THE main course of these investigations, subsequent to the initial work of classification (1 and 2) has been described in three previous publications.

The first communication was made to the British Association at Cardiff in August 1920 (3) and gave the first indications of differential influence of rootstocks upon one or two year old trees both in the nursery and plantation. The differences in the weights, heights, amount of wood growth and in the presence or absence of axillary bloom buds upon the one and two year wood growth of young trees of two scion varieties upon eight rootstocks were so striking that these apple rootstocks were claimed to fall readily into four groups, according to their influence. Whilst the correlation between vigour and early bloom formation was generally obvious at this early age, even then exceptions, such as scions upon Nos. II and V, were apparent, and it was suggested that the figures presented must not be regarded as an ultimate measure. It was pointed out that the constancy and duration of such influences could only be established as the records accumulated year by year and as new graftings and plantings were made.

The second report appeared six years later after it had been given to the British Association Meeting at Oxford in 1927 (4). The measurements of total wood growth, girth of stem, height and spread of tree and number and weight of fruit for the variety Lane's Prince Albert upon sixteen different rootstocks were detailed. Whilst a statistical analysis of the figures confirmed that the widely different stages in vigour and cropping, as typified by the original grouping, were both significant and maintained to the end of the seventh year, it revealed an almost continuous gradation upon other stocks. The comparison of trees of Lane's Prince Albert on rootstocks Nos. IX, II, I and XII were especially emphasized but by this time it had also become apparent that trees of similar size and vigour, upon different rootstocks, not infrequently differed widely in cropping performance. For instance when at seven years old there was no significant difference in the total amount of wood growth made by trees on Nos. IV and XII or VII and I, yet they differed very strikingly in the number of fruits produced.

Up to that time there had been few radical changes in the relative order of vigour of Lane's Prince Albert on different stocks from year to year, and, with few exceptions, the different scion varieties under trial had not suggested any very drastic qualifications. By now, differences in habit of the growth and the position of the fruit buds had been noted. Finally, some figures were presented from four of the subsidiary field trials upon widely differing soils in the South-Eastern area and the claim put forward that a constant *relative* standard of performance may be expected.

The third progress report of these investigations, embodying detailed figures, was given at the Masters' Memorial Lectures of the Royal Horticultural Society for 1929 (5). The additional three years growth data (i.e. to the end of 1928, the trees now being ten years old), upon Lane's Prince Albert, showed that, with the single exception of the trees on No. X rootstock, not only were the original differences maintained but in many cases were becoming more marked, some of the intermediate stocks now standing out with significantly different performance. For the first time measurements of growth and cropping from four other scion varieties: Bramley's Seedling, Worcester Pearmain, Cox's Orange Pippin and Stirling Castle—were presented for comparison with those from Lane's Prince Albert. The differential effect of certain rootstocks—especially Nos. I and II—when combined with different scions was demonstrated for the first time.

Varietal preferences on other stocks, notably Nos. X and XIII, were becoming evident, and it was suggested that different scion varieties responded in different degrees to rootstock influences.

The available data on the subsidiary plots were summarized and shown to support the thesis that on widely different soil types in the South-Eastern area the growth and cropping performance of the same scion varieties on a series of rootstocks was remarkably constant relatively, though the actual size of tree on any particular rootstock differed immensely according to local conditions. Some modifications traced to subsoil conditions and manurial and other cultural treatments were reported and explained.

With the encouragement of the present writer, Mr. R. T. Pearl, of the S.E.A. College, Wye, published a most complete and valuable summary (6) of the East Malling results (1912-31) which renders it unnecessary to review the investigations in greater detail here. However so much additional data have become available since 1928 to illustrate the progress of these studies that a further stage in their development can be said to have been reached. Although the complete picture of the initial plantings will in all probability take another twenty years to fill in, another progress report appears justified.

MATERIAL.

Since the data to be presented are taken entirely from the same plantings as have already been described in detail, little further description of the material is necessary though certain alterations in cultural and other treatments must be referred to and the account will be taken up where it was left off, another six years having elapsed and the trees being sixteen years old.

It should perhaps be emphasized here that, unless otherwise stated, all the trees in the experiment were budded in normal European manner upon the stems of the different rootstocks. The rootstocks were all raised in the Research Station Nursery by methods of layering, from the original material which had been collected from representative European Nurserymen. This material was botanically described (1 and 2) and classified and all those plants which appeared to be morphologically identical were initially grouped under one " type " number and were considered to have been a clone, since the origin and history of the first parent " paradise " plants was generally well known—though their descriptions were usually vague. There is little or no reason—with one possible exception—to believe this action, together with the observations and deductions which prompted it, was mistaken, though in order to prove this, a typical plant from each variety was subsequently selected from which a new clonal race has been multiplied for use in all future experiments.

All the trees referred to in this paper were planted as one-year-olds and formed into more or less open bush trees with four to five initial main branches on a two foot stem. In the subsequent prunings, which were necessary for the normal health and growth of the varieties, certain rule of thumb methods were adopted which ensured that any variety on the whole series of rootstocks received relatively identical treatment and not selective pruning suitable to the individual tree. In the same way, except where otherwise stated, every effort was made to secure uniformity in spraying, manuring and other cultural treatments.

Since the proper planning of experimental plots of fruit trees had as yet received little attention—indeed the necessary data upon which to work were not yet available—the trees were planted in at least two, and wherever possible, four locations, usually in groups of four, upon soil of considerable uniformity which had been uniformly cropped for at least four years prior to the initiation of the experiment.

The data from the Lane's Prince Albert trees have been taken from the second and more considerable planting of 2 acres (Plot 11) made in 1920. This plantation has been kept cultivated uniformly throughout its history and there have been no differential spraying or manurial treatments.

The situation is not quite so simple in the case of the other three scion varieties: Bramley's Seedling, Worcester Pearmain and Cox's Orange Pippin. During the course of the development of these plantations it became necessary to impose changed cultural, manurial or spraying treatments upon the trees, and sometimes, in order to ascertain the optimum treatment, it was necessary to superimpose differential ones.

As there were comparatively large units of Bramley's Seedling and Worcester Pearmain trees—altogether some 2½ acres of these were planted up—it is however, possible to select those areas where the issue is still a clear one of scion response to rootstock effect under standard cultural and manurial conditions. As has already been reported by Hoblyn and Bane (7) the Bramley's Seedling trees were growing so strongly on most rootstocks and the Worcester Pearmains producing such poor quality fruit on the East Malling soil that it was necessary to take drastic action by grassing down. This meant that ultimately remedial measures in the form of differential nitrogenous dressings had to be applied with the result that individual rootstock response to manuring became obvious. However before this had happened, it was decided to grub 50 per cent. of the trees which were too close at 15 feet square, so that fortunately there are records from complete series of Bramley's Seedling and Worcester Pearmain trees up to the end of their sixteenth and fourteenth years respectively, which can be considered without any serious qualifications regarding individual rootstock response to differential treatments.

The records to be discussed in detail come from the Bramley's Seedling trees from Plot 12 and the Worcester Pearmain from Plot 13. The units of Bramley's Seedling—usually eight trees—were from the first divided equally into pruned and unpruned series, but since the leader tipping on this variety is invariably light and of short duration and spur pruning was not practised, the differential treatment gave little significant effect especially since a standard method of thinning all trees was adopted from 1927 onwards.

Although the Bramley's Seedling trees growing on Plot 12 were not removed until five years after the plot had been grassed down they received no differential manurial treatments, merely acting as "buffer" trees between the treated Worcester Pearmain for two seasons prior to grubbing. It was especially remarked at the time how surprisingly local appeared to be the effect of the nitrogen dressings applied to the Worcester Pearmain trees, though individual branches of the Bramley's Seedling trees showed only too clearly, if and when they were "robbing" the intermediate manured plots. Whilst any serious influence on normal rootstock effect from selective manuring did not become apparent, a close study of the records later suggests that the Bramley's Seedling trees did in fact benefit generally from the presence of nitrogen applied to the neighbouring trees.

The effect *per se* of the grassing down between the Spring of 1930 and the Autumn of 1934 must also be considered. It has already been reported elsewhere that nitrogen deficiency in these trees did not become apparent before the Summer of 1932, but that from that time onwards the trees on the more dwarfing rootstocks showed generally an earlier yellowing and fall of leaf than those on the most vigorous ones. However, a close examination of the records from year to year over this period (1930-1934) fails to show any relative inconsistency in the behaviour of these trees so that the data can fairly be regarded as illustrating their normal trend had they remained under cultivation, provided that it is remembered that certainly most, and probably all, of them were not displaying their optimum performance under the grass.

The Worcester Pearmain trees on Plot 13 were all pruned to a standard method up to 1925 ; subsequently, with certain exceptions, they were treated according to the needs of the trees as a whole, being tipped in 1928, 1929 and 1931 and in other years merely thinned. The trees on six stocks, Nos. I, II, IX, X, XII and XVI were tipped every year until 1929 to facilitate the continuance of wood measurements. Although the plot was grassed down in the Spring of 1932, the Worcester Pearmains were removed the same Autumn, before the grass had become sufficiently established to have any effect in the initial year.

As 50 per cent. of the Bramley's Seedling and Worcester Pearmain trees interplanted at 15 feet square were grubbed to allow for the normal development of the remainder without interference, it is now possible to give something in the nature of a total tree weight of 123 fourteen-year-old Worcester Pearmain trees on a range of seventeen different rootstocks grubbed in the Spring of 1933, and a similar number of Bramley's Seedling on nineteen root systems grubbed at sixteen years old in the Winter of 1934.

The Cox's Orange Pippin trees were planted in February 1922, unfortunately on a less complete range of rootstocks—as it was then thought on this soil to be impossible to grow this variety at all—with the variety Stirling Castle interplanted on a somewhat similar range as a pollinator. All the trees were pruned and manured on a standard treatment, but since no satisfactory method of Scab and Canker control had as yet been established for the growing of these varieties upon the Malling Series of the Lower Greensand, spraying had to be made a main issue on this experimental planting.

The Stirling Castle variety proved so susceptible to injury, even from proximity to the spray fluids being used, that it was soon abandoned for rootstock investigation purposes and, during the last few years, has been systematically top- and stub-grafted to Worcester Pearmain for pollination purposes.

The spraying treatments were arranged on the Cox's Orange Pippin trees, with the definite object in view of uniformly spreading the spray treatments and controls throughout every stock-scion combination. The trees on Nos. I, II, IX and XVI can readily be compared throughout since they received a single pre-blossom spray only up to the end of 1930, and no damage of any consequence was recorded. Whilst some trees on Nos. V, XIII, XV, III and IV cannot strictly be compared with the former group owing to the damage caused by the use of Bordeaux Mixture in the early years, it is possible from the control trees of this group to check up whether any serious qualification is necessary in making comparisons. A careful examination of the common controls shows that, with the possible exception of the trees on No. XV, the general rootstock-scion relationships have not been disturbed.

After 1930 the experiments were similarly extended to include the trees on Nos. I, II, IX and XVI, and the various differential sprayings were uniformly distributed.

In the earlier years at any rate, the experimental sprays were known to cause leaf and fruit drop and so undoubtedly reduced the potential total crops of Cox's Orange Pippin. But until a satisfactory schedule could be found this was inevitable, and in any case the unsprayed control trees suffered equally badly—if not worse—through the damage caused by Apple Scab and Red Spider.

Scion Rooting.

In considering the character of the material from which the data have been collected, this phenomenon must not be ignored. Although horticultural tradition emphasized the need of burying the union between scion and " paradise " rootstock below ground level, an attempt was made to plant these experimental trees with their unions at or just above ground level so that these unions could be kept under observation and, if necessity demanded, be subsequently earthed up. Unfortunately, sufficient allowance was not made both for the normal sinking of the trees in the soil after planting and for the effect of the annual ploughings and cultivations which tend to throw the earth up towards the tree rows. With few exceptions the bases of the scions are now all below ground level. Although a considerable number of Lane's Prince Albert trees have been excavated, in no instance has there been any sign of scion rooting. Of the 123 Worcester Pearmain trees grubbed no serious case of scion rooting was to be found though it was reported as slight on two trees on No. IX stock. Nor would the general record of the trees of these two varieties suggest any abnormal behaviour. However, the case of Bramley's Seedling is rather different. At quite an early stage, it became evident that some of the very dwarfed trees of this variety upon Nos. VIII and IX rootstocks were beginning to grow exceptionally vigorous and upright. Scion rooting was suspected, and these suspicions were partially confirmed by careful examination after the soil had been cleared from around the union. It would appear that between 1925-28, six of the eight trees upon No. IX established themselves sufficiently on their own roots markedly to affect vigour and type of growth—and therefore fruiting. When the trees were excavated in 1934 seven of the eight trees were confirmed to be established on their own roots and, in at least three of these cases, the original No. IX stock roots were completely dead or dying. All three trees on No. VIII roots were similarly affected. Despite the fact that the data from these trees very generally fall into line with those of other varieties upon these rootstocks, in placing them in a class by themselves as the most dwarfed and early cropping trees, the figures cannot claim to present the normal performance of these combinations of stock and scion, though it is impossible to say exactly how long and in what degree this scion rooting has exerted any considerable influence upon these Bramley's Seedling trees.

Three trees of Bramley's Seedling on No. II and one on No. VII rootstock were also found to be scion rooted though not to so considerable an extent as the trees on Nos. VIII and IX. However, a close examination of the individual tree records suggests that in 1932 and 1933 these trees were behaving slightly differently from the remainder in their respective groups in the matter of girth increment. Probably therefore scion rooting was just becoming an important factor at the time the trees were grubbed.

As yet there is no evidence as to the propensity of Cox's Orange Pippin to establish itself on its own roots but there is up to the present nothing very exceptional in the behaviour of the trees to arouse suspicions.

ROOTSTOCK EFFECT UPON TREE GROWTH.

MEASUREMENTS OF VIGOUR.

(*a*) *Total wood growth.*

Unfortunately it has only been possible since 1928 to maintain this record to date upon one variety—Lane's Prince Albert—upon eight different rootstocks—Nos. I, II, IV, VII, IX, X, XII and XVI. Even here upon the larger trees it has been necessary to employ a sampling technique, based upon records of wood pruned off, only a small proportion of the trees being measured entirely. This has been done on eighty-nine trees. Complete wood measurements have been carried out annually on a further seventy-seven smaller trees. Wood measurements were maintained on the Worcester Pearmain trees for two more years on rootstocks Nos. IX, II, I and XVI, whilst a method of estimating wood growth from weights of prunings and sample lengths has been carried out for three years (1932-34) for Cox's Orange Pippin on the same four stocks.

(*b*) *Cross Section of Stem. Thickness of Trunk.*

The thickness of the trunk is represented by the area of its cross section. This is calculated from a girth measurement at a fixed height, it being assumed that the cross section is circular. For this purpose girth is still recorded and affords a quicker and more accurate result than a measurement of diameter. Cross section is now used for comparative purposes in preference to girth, since a real picture of the actual amount of stem thickening is obtained when increments from year to year are compared.

(*c*) *Height and Spread of Branches.*

Although this is a somewhat crude form of record, it is the only one which gives an idea of the tree's stature and the space occupied thereby at any particular time. The " average " height is recorded and the spread of branches measured annually through the tree in two fixed directions. It is recognized that these observations may be affected by weight of crop as well as by habit of growth, and unavoidable prunings to admit of tractor cultivation.

(*d*) *Total weight of Tree.*

Whilst Rogers and Vyvyan have already given some figures (8) comparing the total weight of branches, stem and roots of some eight carefully excavated ten- and eleven-year-old trees of Lane's Prince Albert on Nos. I and IX roots at East Malling, so far the main planting of this variety remains intact.

In the case of the Bramley's Seedling and Worcester Pearmain trees grubbed in 1933 and 1934 the top soil was removed and the main roots severed at 3 feet (·91 metre) from the trunk in the case of the Bramley's Seedling and at 2 feet (·61 metre) in the case of the Worcester Pearmain. The methods used and the analysis of these " skeleton " root systems are described in detail by Bane, Beard and de Wet (9). Beakbane and de Wet excavated three of the sixteen-year-old Bramley's Seedling trees in much greater detail, digging 4 metres square and a metre deep as reported (10).

Thus one-half of the Bramley's Seedling and Worcester Pearmain trees—usually eight trees of each variety on each rootstock—now present something in the nature of a complete picture of the root, stem, branch and fruit performance up to fourteen and sixteen years respectively.

(*a*) *Measurements of Total Wood Growth.*

A record of total wood growth to the end of 1925 from the series of Lane's Prince Albert upon the complete series of rootstocks has already been published. These records were taken for a further two years and then discontinued on all but eight rootstocks after the Winter of 1927. With three exceptions, all the trees had produced approximately two-and-a-half times as much wood growth in these two years, i.e. the eighth and ninth seasons, and so their relationship remained unaltered. Trees on Nos. VII and X only about doubled their total wood growth, and those on No. IV did little more. Whilst trees on Nos. VII and X now definitely lost their positions and fell back in the list of total wood measurements with only 335 and 258 metres of growth, those on No. IV still appeared third on the list with an average of 473 metres to their credit, as compared with 557 and 580 metres for those on Nos. XVI and XII respectively.

This tendency becomes more marked when the measurements of the selected trees, which have been maintained to date, are studied.

In the Tables which follow those stocks whose differences are not statistically significant are bracketed together. In order to save space the standard errors are omitted. In all cases however these have been calculated and are the basis on which these significances are judged.

The means of any pair of stocks are said to differ significantly if they differ by more than twice the standard error of their difference. In such cases the odds are at least twenty to one against that difference being merely due to chance.

TABLE I.

Total Wood Growth of Lane's Prince Albert on Various Rootstocks, in metres.

Stock.	1928 (10 years).	Stock.	1934 (16 years).
IX	137	IX	226
X	327	X	617
II	389	VII	641
VII	425	IV	1,030
I	548	II	1,210
IV	620	I	1,381
XVI	773	XVI	2,068
XII	810	XII	2,560

Table I shows that in the intervening six years, the trees on Nos. II and XII have approximately trebled their total wood growth ; those on Nos. I and XVI have increased almost exactly two-and-a-half times ; those on Nos. IX and X have not quite doubled the 1928 total, whilst the trees on Nos. IV and VII have added only about half as much growth to their totals.

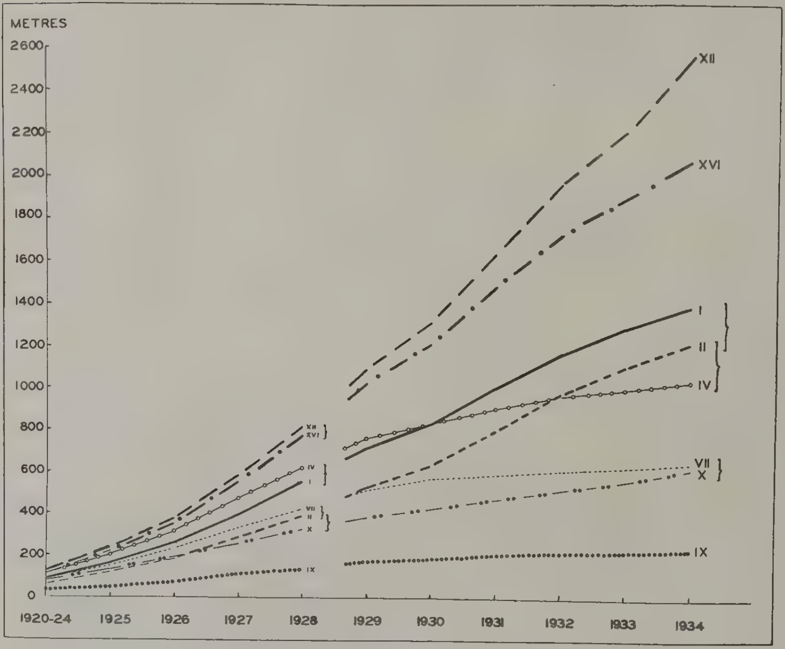
The development of this change is most conveniently shown in Diagram A, where the accumulated total growths are compared over a ten year period—starting in 1924 when the trees were already six years old.

Whilst at sixteen years old the Lane's Prince Albert on No. XII are still growing away from all the others, those on XVI have by comparison definitely begun to slow down during the last two years. Though the trees on Nos. I and II have maintained a fairly constant relationship, it now becomes evident that during the past three years those on No. II have been steadily catching up those on No. I. From 1928 onwards the trees on No. I were growing faster than those on No. IV, which, from the 1930 season onwards, fell off rapidly in wood growth. Trees on No. VII actually began a similar process a year earlier, and fell away very rapidly from 1929 onwards.

Lane's Prince Albert on No. X started to fall away as early as 1926; and though until 1930 these trees were considerably smaller than those on No. VII, since that date the difference between them has gradually diminished.

From 1928 onwards the trees on No. IX have only added some 80 metres of new wood growth.

Diagram A.



Total accumulated Wood Growth of Lane's Prince Albert on 8 different rootstocks (from 6 to 16 years).

How far these changes can be correlated with the cropping of the trees will become more obvious when their annual growth performance can be studied in the light of the annual crop (see Diagram C).

In the earlier reports, it was apparent that the largest trees (on No. XII) had, at seven and ten years respectively, made approximately five and six times as much wood growth as the smallest ones (on No. IX); at sixteen years they have made approximately ten times as much. Meanwhile trees on such stocks as Nos. XVI and IV and Nos. I and VII, which after seven seasons showed no significant differences in wood growth in their very vigorous and vigorous groups, have after sixteen seasons very definitely parted company. The trees on No. XVI have since made double the growth of those on No. IV, and those on No. VII are surpassed by the trees on No. I by an approximately similar amount.

Even if there were only the data for a single variety, Lane's Prince Albert, to be considered, it would be unwise at the moment to attempt from a theoretical point of view to revise the old grouping method. Although the "dwarfing" (*a*) and "very vigorous" (*d*) groups are wider apart than ever and although it appears that the big gap between trees on Nos. IX and II may be at least partially filled by those on Nos. X, VII and IV which promise to remain more truly "semi-dwarf" (*b*), the final relationships between trees on Nos. II, I and even No. XVI cannot yet be determined with any certainty.

When the data from other varieties under varying cultural and manurial treatments have been considered this will become even more evident, though the desirability (not only from a practical point of view) for maintaining the group idea will remain obvious, since certain varieties of rootstocks respond to certain treatments in one direction and other varieties in another.

Unfortunately the mechanical difficulties of maintaining these total wood measurements make it impossible to present complete records for the other varieties—Bramley's Seedling, Worcester Pearmain and Cox's Orange Pippin. However the further two years records on Worcester Pearmain on four of the leading rootstocks show in Table II that the significant differences which were apparent in 1928 were not only maintained but accentuated.

TABLE II.

Total Wood Growth of Worcester Pearmain on Various Rootstocks, in metres.

Stock.	1928 (10 years).	1930 (12 years).
IX	189	197
I	281	410
II	310 ⎫	527
XVI	317 ⎭	764

The bracket indicates an insignificant difference.

Finally it has been found possible by estimation from the weight of a metre of new wood (determined annually by sampling) to arrive at the approximate length of new growth removed in pruning, from the weight of the prunings. This method is quite suitable for a variety which is regularly spur pruned like Cox's Orange Pippin. With such a variety either the whole or a large proportion of every spur growth over a certain length is removed and a definite proportion of the leaders. It is found that this growth removed is very nearly proportional to the total wood growth for the year on each rootstock.

The results of this method as tested upon this variety are given in Table III.

TABLE III.

Total Wood Growth cut off in Pruning for three years on Cox's Orange Pippin as estimated from Weight of Prunings and sample length.

	1932 (12th year).		1933 (13th year).		1934 (14th year).	
	Weight of metre of new wood gms.	Length in Metres.	Weight of metre of new wood gms.	Length in Metres.	Weight of metre of new wood gms.	Length in Metres.
IX	14·8	**50**	11·8	**52**	10·8	**28**
I	13·5	**80**	10·2	**60**	10·4	**23**
II	14·7	**161**	11·1	**184**	10·4	**127**
XVI	14·5	**254**	12·6	**289**	11·3	**278**

This method is of recent development and such is the importance now attributed to this aspect of vigour that a further method using sample branches is being tried for use on trees not spur pruned.

More recently still Vyvyan (11) has developed a method of making a summer growth census which it is hoped will be of great value in throwing light on the time of formation, amount and nature of this annual wood growth.

In the meantime reliance has been placed on more simple measurements of vigour.

The relationship between these and total wood growth, as recorded upon Lane's Prince Albert, affords a measure of their value in giving a true picture of the tree's growth.

(*b*) *Cross Section of Stem. Thickness of Trunk.*

Diagram B shows the accumulated data for this record over the same ten year period and on the identical trees of Lane's Prince Albert on the same eight rootstocks used in Diagram A.

Whilst the actual order of vigour—with one insignificant difference (the trees on Nos. X and VII)—remains the same, it is obvious that the two diagrams do not tell quite the same story and that there must be other factors related to stem increment besides annual increase in wood growth. If the amount of wood growth per unit of cross section of stem be calculated at the end of 1934, it will be found that this varies quite considerably. This is shown in Table IV.

Hoblyn and Bane (7) have already shown increment of Cross Section of Stem to be related both to nutritional and cropping differences, and when the data for total weight of tree come to be considered in relation to this measure it will be seen that trees upon certain rootstocks (especially Nos. II and V) have

TABLE IV.

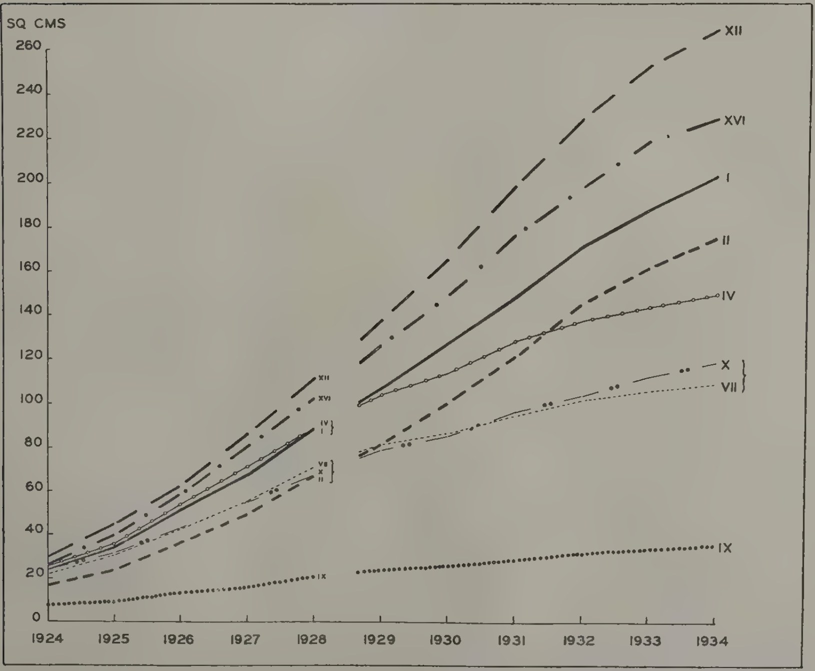
Amount of Wood Growth per unit of Cross Section of Lane's Prince Albert, sixteen years old.

Rootstock ..	X	VII	IX	I	II	IV	XVI	XII
Wood Growth	5·2	5·9	6·5	6·8	6·9	6·9	9·1	9·5

markedly thicker trunks in proportion to total growth and increment of the tree than would be expected in comparison with trees on the majority of rootstocks.

Unfortunately subsequent to 1927 this is the only detailed measure of vigour which it has been possible to maintain on the complete series of Lane's Prince Albert trees. However, if the above qualifications are kept in mind and it is remembered that these trees have throughout been treated alike culturally and manurially, the figures for cross section of stem at sixteen years

DIAGRAM B.



Total accumulated Cross Section of Stem of Lane's Prince Albert on 8 different rootstocks (from 6 to 16 years).

TABLE V.

Area of Cross Section of Stem in square cm. of Lane's Prince Albert (Plot 11) on different Rootstocks (sixteen years old, 1934).

Stock ..	IX	VIII	H	VII	X	III	XI	IV	XIV	V	A	II	XV	F	XIII	I	VI	OF5	C	XVI	E	XII
No. of trees	(20)	(8)	(2)	(19)	(20)	(19)	(9)	(9)	(8)	(20)	(5)	(20)	(18)	(4)	(20)	(20)	(20)	(4)	(4)	(20)	(4)	(29)
Cross Section	35	56	83	109	118	136	142	149	163	169	169	175	176	185	189	203	214	220	224	228	258	269

TABLE VI.

Area of Cross Section of Stem in square cm. of Bramley's Seedling (Plot 12) on different Rootstocks (sixteen years old, 1934).

Stock ..	VIII*	IX	X	VII	IV	III	V	XI*	XV	XIV*	XIII	VI	II	I	XVI*	E*	OF5*	F*	XII	A	C	H
No. of trees	(3)	(8)	(8)	(8)	(8)	(8)	(8)	(4)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(3)	(4)	(4)	(8)	no trees		
Cross Section	139	142	225	228	242	256	291	299	301	304	305	308	310	311	349	361	368	384	387			

* All the trees in these series were lightly tipped until 1926. See text. Possibly these values are slightly smaller than would have been the case if the trees had been left untipped as in all the remaining series. Hence the figures are not strictly comparable.

TABLE VII.

Area of Cross Section of Stem of Worcester Pearmain (Plot 13) on different Rootstocks—fourteen years old at the end of 1932.

Stock ..	IX	VII	VI	X	E	I	XIV	IV	XIII	OF5	XI	V	III	XV	XVI	II	F	XII	VIII	A	C	H
No. of trees	(8)	(8)	(8)	(8)	(1)	(8)	(3)	(8)	(8)	(6)	(7)	(8)	(8)	(8)	(8)	(8)	(3)	(8)	No trees			
Cross Section	45	86	104	110	111	112	122	122	123	141	147	149	159	160	162	163	173	182				

old presented in Table V afford a generally reliable guide for placing the rootstocks in an order which represents the vigour of the trees.

In this Table V, the complete range of rootstocks originally under trial is included, although in many cases the unit of trees is very small. However most of these rootstocks have been distributed at one time or another for experimental purposes, and they include such important and valuable vigorous stocks as Long Ashton OF 5 and East Malling C. It may be recalled that H, A, F, C and E are the vegetatively raised progeny from a range of seedling French Crabs, selected at East Malling for showing " burr knots " (root initials) as quite young plants, whilst OF 5 was the original Free stock, vegetatively raised at Long Ashton Horticultural Research Station by John Ettle to ensure uniform trees in the trial cider orchards, as far back as 1903.

This Table gives a good picture of the decline in vigour of the trees upon Nos. VII, X, IV, XV and XIII which has actually taken place since the last wood measurements for the complete series were made, although trees on No. XII roots have already been shown to be making ten times the growth of those on No. IX, yet the trunks of the former are at present approximately only eight times as large.

A similar measure of vigour is available for the series of Bramley's Seedling, Worcester Pearmain and Cox's Orange Pippin. The issue however in these cases is not always quite so simple as that on the two acres of Lane's Prince Albert (Plot 11) under review owing to the other factors affecting tree performance mentioned in the general discussion of the material.

In Table VI areas of cross section of stem of the trees at the time of grubbing on the complete range of rootstocks worked with Bramley's Seedling are presented.

Before attempting to make a comparison with Table V, it will be most convenient to give similar data for the Worcester Pearmain trees from Plot 13 and the Cox's Orange Pippin from Plot 32.

Table VII shows the areas of cross section of stem at the time of grubbing of the complete range of rootstocks worked with Worcester Pearmain and planted on Plot 13.

Despite the disturbing factors already referred to in relation to spraying treatments the data presented in Table VIII are remarkably suggestive and give a not unfair comparison of the relative performance of a series of Cox's Orange Pippin apple trees, grown in the early years under anything but optimum conditions of pest and disease control.

Since, unfortunately, the same number and variety of combinations of stock and scion are not always available for comparison in Tables VI, VII and VIII, the true situation is best shown by comparing the behaviour of these four very different yet representative scion varieties upon the ten most important rootstocks amongst those originally selected. The actual order of vigour, based on thickness of trunk is shown in Table IX.

It at once becomes evident that the scion varieties are all responding similarly, at least relatively, to the influence of five rootstocks—Nos. VII, IX, X, XII and XVI—and apparently in a lesser degree to No. IV. This last case really only helps to illustrate what is happening. The trees of Worcester Pearmain and Cox's Orange at fourteen years appear more vigorous on No. IV

TABLE VIII.

Area of Cross Section of Stem in square cm. of Cox's Orange Pippin on different Rootstocks, fourteen years old in 1934 (Plot 32).

Stock ..	IX	I	III	X	VI	V	IV	XIII	II	XV	XVI
No. of trees	(16)	(16)	(16)	(15)	(14)	(16)	(8)	(16)	(16)	(8)	(16)
Cross Section	41	62	66	84	85	94	97	97	100	111	118

Note.—XIII is significantly different from X, the mean (of sixteen trees) being more reliable than that of IV which is only based on eight trees.

TABLE IX.

Order of Vigour as indicated by Area of Cross Section of Stem on Four Varieties on Ten Rootstocks.

	Years old	I	II	IV	V	VI	VII	IX	X	XII	XVI
Lane's Prince Albert ..	16	7	6	4	5	8	2	1	3	10	9
Bramley's Seedling	16	8	7	4	5	6	3	1	2	10	9
Worcester Pearmain ..	14	5	9	6	7	3	2	1	4	10	8
Cox's Orange Pippin ..	14	3	8	7	6	5	None	1	4	None	9

TABLE X.

Comparison of Stoutness of Stem of Four Varieties upon Four Rootstocks.

Stock.	Lane's Prince Albert. (16 years).	Bramley's Seedling (16 years).	Worcester Pearmain (14 years).	Cox's Orange Pippin (14 years).
No.	sq. cm.	sq. cm.	sq. cm.	sq. cm.
IX	35	142	45	41
X	118	225	110	84
IV	149	242	122	97
XVI	228	349	162	118

than do Bramley's Seedling and Lane's Prince Albert at sixteen years. However, if the increment of cross section be studied from year to year it will be found that the trees of Cox's Orange Pippin on No. IV have been falling off markedly ever since 1931—a process likely to be accentuated with increased cropping—and those of Worcester Pearmain showed a similar tendency up to the time they were grubbed.

Actually of course the stem thickening made by each one of these scions on any one of this series of stocks may vary very strikingly. Compare, for instance, the actual stoutness of stem of these four varieties on four rootstocks as shown in Table X.

Obviously any particular scion is only *relatively* more vigorous or more dwarf upon any given rootstock. Bramley's Seedling on No. XVI has approximately one-and-a-half times the trunk of Lane's; twice that of Worcester Pearmain and three times that of Cox's Orange Pippin on the same stock, yet they are all by comparison very vigorous trees of their particular variety.

Whilst the development of the experiments in the past few years suggests that it would be rash to make further prophesies, it would seem that a definite trend of growth and cropping may be now expected from these six rootstocks (i.e. Nos. IX, VII, IV, X, XII and XVI) though it is still possible that some scion varieties may be found to qualify this. So far upon different soils in the South-East of England and under different manurial treatments at East Malling this trend has remained unaltered. The data for the further experimental plantings of Bramley's Seedling (Plot 13) and Worcester Pearmain (Plot 12) on the same range of rootstocks which are undergoing differential manurial treatments, will not be presented here. At present they can be more appropriately discussed from the nutritional standpoint. However, it may be mentioned that although the trees of both varieties on these six stocks are equally divided so that they receive various amounts of nitrogen, the trunk thickening, whilst differing for those different manurial treatments, remains in the same relative order as is shown in Table IX so far as rootstock is concerned.

The position of the remaining four rootstocks—Nos. I, II, V and VI in Table IX suggests a far more complicated story, which as yet has not fully worked itself out. It seems evident that there are definite scion partialities which are becoming apparent as the result of a strikingly different response to rootstock influence. The trees of Lane's Prince Albert on No. VI (214 sq. cm.) do not differ significantly in thickness of trunk from those on the vigorous No. XVI (228 sq. cm.), while those on No. I (203 sq. cm.) are not far behind, yet they are both significantly stouter than those on Nos. II and V (175 and 169 sq. cm.). In the case of Bramley's Seedling, trees on all four stocks whilst bracketed together (291, 308, 310 and 311 sq. cm.) are still significantly smaller than those on No. XVI (349 sq. cm.). In the case of Worcester Pearmain the trees on Nos. VI and I (104 and 111 sq. cm.) have comparatively slender stems compared with those on Nos. V and II (149 and 163 sq. cm.), whilst trees on this last stock have trunks as thick as those on No. XVI (162 and 163 sq. cm.). The position with the scion variety Cox's Orange Pippin is most startling of all, for here the trees on No. I (62 sq. cm.) appear as the second most dwarfed on the table, whilst those on Nos. VI and V (85 and 94 sq. cm.) are bracketed together in an intermediate position and those on No. II (100 sq. cm.) are only just significantly less stout than the very vigorous ones on No. XVI (118 sq. cm.).

It will be remembered that the early results pointed generally to the vigorous growth of the varieties tried upon Nos. I and VI and the earlier formation of axillary fruit buds on No. II. The examination of the full cropping records to date will however demonstrate the much better fruiting performance of the trees on No. I as compared with those on No. II in three of the four cases. Even in the case of Cox's Orange Pippin, where the trees on both stocks had cropped about equally until 1932, it must be remembered that relatively the trees on No. I had cropped much more heavily for size of tree, so that heavy cropping and checked growth may be the explanation. The dwarfing nature of No. I rootstock when in combination with scions of Mackintosh Red has also been reported to the writer by Dr. J. K. Shaw who is testing out some of these rootstocks in Massachusetts. On the other hand, Dr. Burt has reported that, in the United Provinces, India, the variety Tomkins King has made fine trees on No. I which were more vigorous than those on No. II at six years old.

Again, whilst all the scions tested on No. V rootstock have been comparatively poor in cropping, yet their vigour, as expressed by trunk thickness, varies considerably from variety to variety.

It may here be recalled that on the "starvation acre" (Plot 14) where different remedial dressings were subsequently applied, as already described by Hoblyn and Bane (7), these four rootstocks proved of individual interest both in the length of time they took to show manurial deficiencies and in the manner and degree in which they responded more or less strikingly and even individually to these remedial measures. Incidentally trees on No. III were grouped with the rest of the Doucin types (Nos. II and V) as being affected earlier and more markedly than those on Nos. I and VI. In Tables V—VIII the position of No. III is again variable, the trees of Lane's Prince Albert and Cox's Orange are weak, those of Bramley's Seedling and Worcester Pearmain medium and strong respectively. Finally, it is noteworthy that trees on No. IV rootstock under all treatments maintained their reputation for heavy cropping and showed manurial differences less clearly than those on the other stocks under experiment.

Since it has been proved then that these rootstocks respond in a greater or less degree to certain manurial treatments, it is only reasonable to suppose that they will supply more or less efficiently those particular requirements of individual scions which demand their own peculiar nutrition. Whilst this fact may complicate the issue from the practical point of view and delay the time for drawing final conclusions, it merely goes to emphasize the vital importance of extending the knowledge of stock influence in relation to the scion partialities of at least the other commercial varieties of apple. Of the less important rootstocks, No. VIII appears to be invariably linked with No. IX; trees on Nos. XIII and XV appear to be moderately vigorous with Bramley's Seedling and Worcester Pearmain, but distinctly more so with Lane's Prince Albert and Cox's Orange.

The very promising vigorous Long Ashton OF 5 and East Malling Crab F (368 and 384 sq. cm.) appear together as very stout stemmed with Bramley's Seedling, but whilst Lane's Prince Albert makes a stouter tree on OF 5 (220 as against 185 sq. cm.), Worcester Pearmain does the reverse (141 sq. cm. as against 173 on Crab F).

Whilst trees on Nos. XI and XIV, both of medium vigour, are not separated by any significant differences in stem thickening, it will be found later that they differ strikingly in fruiting capacity.

Finally, Tables V to VIII deserve detailed consideration from the point of view of the response of each individual variety to the whole series of rootstocks.

Taking Bramley's Seedling (Table VI), although there are actually only two definite breaks—i.e. those between trees on Nos. IX and X and again between those on Nos. I and XVI, there remain nevertheless four pretty distinct groups so far as vigour expressed in terms of thickness of trunk is concerned. The first group containing Nos. VIII and IX stands quite apart, despite the gradual rooting of the scion upon these two rootstocks. A second group at the moment contains Nos. X, VII, IV and III. The third, the most inclusive group in the whole series, embraces eight rootstocks, Nos. V, XI, XV, XIV, XIII, VI, II and I. The fourth and most vigorous group contains trees on Nos. XVI, E, OF 5, F and XII. Again with Worcester Pearmain as scion (Table VII), No. IX stands in a group apart, but there are intermediate stocks connecting up all the other groups. Whilst Nos. VI, X, I, IV, XIV and XIII appear to form a large second group, they are closely connected with Nos. OF 5, XI, V which suggest a third group. The fourth and most vigorous group contains Nos. III, XV, XVI, II, F and XII, but is connected with the earlier group of Nos. OF 5, XI and V up to the trees on No. II.

The Lane's Prince Albert trees (Table V) afford an interesting and instructive change. Actually the performance of the dwarf trees on Nos. IX, VIII and Crab H is at present significantly different in each case, but the small number of trees on Crab H at any rate justify its omission from this review, nor does the position of the trees on No. VIII—six of which are on headlands—allow of the difference being taken too seriously. Nos. VII and X form a second group to themselves, whilst a third is formed by the trees on Nos. III, XI, IV and XIV. A fourth group might include Nos. V, A, II, XV and F, though it is connected up on either hand with less and more vigorous groups. Trees on Nos. XIII and I might form a fifth group, though they are closely followed by those on Nos. VI, OF 5, C and XVI in a sixth group. The trees on E and No. XII at the moment stand apart as a very vigorous seventh group.

The smaller series of rootstocks worked with Cox's Orange Pippin (Table VIII) suggests yet another possible contrast resulting from scion partialities. As usual the trees on No. IX form the first group to themselves ; a second independent group consists of Nos. I and III, after which there is a continuous overlapping over a not very wide range of areas (84 to 118 sq. cm.). Whilst Nos. X, VI, V, IV and XIII might form the third group and Nos. XV and XVI the fourth, these two are closely connected by the intermediate trees on No. II.

This aspect of the investigation should sufficiently emphasize the qualifications that different scions are likely to place at any one time on any method of grouping based on any one factor such as vigour. It also illustrates the inadvisability of losing sight of the idea of grouping altogether.

(c) *Height and Spread of Branches.*

This measurement of vigour is in the main of practical importance, since, of necessity, it can only be very approximate and will even vary from year to year in the same tree according to the crop borne and the exigencies of " dehorning " to allow for tractor cultivation.

It is perhaps sufficient here to illustrate the extreme differences existing at present in the four varieties in Table XI.

TABLE XI.

Range of Spread and Height of Four Varieties upon Complete Series of Rootstocks.

The average spread of Lane's Prince Albert ranges from 3 metres on No. IX to 6·3 metres on No. XII.

The average spread of Bramley's Seedling ranges from 5·5 metres on No. IX to 7·4 metres on No. XII.

The average spread of Worcester Pearmain ranges from 3·3 metres on No. IX to 4·1 metres on No. XII.

The average spread of Cox's Orange Pippin ranges from 2·6 metres on No. IX to 3·6 metres on No. IV.

The average height of Lane's Prince Albert ranges from 1·6 metres on No. IX to 4·5 metres on No. XII.

The average height of Bramley's Seedling ranges from 4·1 metres on No. IV to 6·1 metres on No. XVI.

The average height of Worcester Pearmain ranges from 2·7 metres on No. IX to 5·0 metres on No. XII.

The average height of Cox's Orange Pippin ranges from 2·6 metres on No. IX to 3·9 metres on No. XVI.

Actually the few trees on Crab E are slightly taller than the Lane's Prince Albert on No. XII and the Bramley's Seedling on No. XVI—the only two comparisons available—which suggests a possible significant difference in shape.

The study of area of cross section of stem, or even total weight of tree, alone, may be misleading for although trees on particular stocks may not differ significantly for either of these factors, the type of growth and shape of tree may be very different. This is suggested to some extent by the ratio of spread to height which is given in Table XII for three varieties—themselves of very different natural habit—on a selected series of rootstocks to illustrate the point.

TABLE XII.

Ratio of Spread to Height in three Varieties on Rootstocks.

	IX	VII	X	IV	I	II	XVI	XII
16-year-old Lane's Prince Albert ..	1·82	1·80	1·68	1·61	1·51	1·54	1·55	1·41
16-year-old Bramley's Seedling ..	1·21	1·30	1·30	1·44	1·39	1·35	1·17	1·29
14-year-old Worcester Pearmain ..	1·20	0·99	0·96	0·97	0·95	0·83	0·82	0·82

The shape and habit of growth of a variety on a number of individual rootstocks is in actual fact often so characteristic that they cannot fail to be recognized at sight by those associated with these investigations. The most extreme examples of rootstock effect on shape and habit are the trees on No. X. The trees, whilst making few lateral shoots, grow upright and slender in the early years, and always appear to be sparsely furnished with growth and foliage. Whilst this characteristic is most striking with Bramley's Seedling, it is noticeable not only with the varieties dealt with in this report but also with a number of other scions used in an investigation of rootstocks suitable for half standard trees. Trees on No. XIII possess the same characteristic in a modified form.

The series of photographs of sixteen year old Worcester Pearmain trees—taken from the duplicate Plot 12—really illustrate the remarkable differences in the habit of growth, range of vigour and state of maturity of that variety on a representative range of rootstocks. Figure 1 shows a typical tree on No. IX, comparatively spreading for the variety. It is still making a moderate extension though little lateral growth, even growing in grass and after a season of drought.

Figure 2, Worcester Pearmain on No. VII, is still very broad in comparison with height of tree and is still making a little lateral as well as extension growth.

Figure 3 illustrates well how the early vigour of the trees on No. IV has tended to keep the tree more upright, yet the heavy cropping has very definitely checked new growth. Figures 4 and 5 show the much more typical upright Worcester Pearmain growth on Nos. II and I; these trees are still making plenty of extension and lateral growth. Figure 6 illustrates well the exaggerated tendency to upright growth on the very vigorous No. XVI rootstock with a still excessive amount of new lateral growth. These contrasts could be equally well illustrated from the other varieties, but Worcester Pearmain with its naturally compact habit is the easiest subject for photographing in the plantation.

(*d*) *Total Weight of Tree.*

Time alone can make it possible to give the final picture of the declining tree. At the present time (up to seventeen years) none of the combinations of varieties and rootstocks under observation show any definite signs that the trees have passed their zenith, and, although fortunately it is possible to present a probably unique series of figures giving some idea of the complete performance of a considerable number of trees, it must always be borne in mind that these trees were only in their prime—at fourteen and sixteen years—if indeed those on the more vigorous stocks can even now be claimed to have reached that point.

During 1928 and 1929 Rogers and Vyvyan (32) very carefully excavated eight ten year old trees of Lane's Prince Albert from the original planting (Plot 10). Their figures for total weight of tree are worth recalling and summarizing in Table XIII because as much of the root system as possible was included and the weights afford a more exact picture of the different parts of the tree than it has been possible to give for Bramley's Seedling and Worcester Pearmain under the later grubbing technique. This rough method, already referred to, had, of necessity to be employed on Plots 12 and 13 so as not seriously to disturb the roots of the remaining trees. The total weights of trees of Lane's Prince Albert on four representative rootstocks are summarized below.

TABLE XIII.

Average Total Weight in Kilograms of Two Trees of Lane's Prince Albert (ten and eleven years old) when excavated on each of four rootstocks.

	IX*	II	I*	XVI
Weight of Branches and Trunk ..	13·1	34·1	33·1	67·8
Total Root Weight	5·4	15·2	16·3	27·6
Total	18·5	49·3	49·4	95·4

* The figures are not strictly comparable because :
The two trees on IX were ten years old,
One of the trees on No. I was ten years old.

To the figures in Table XIII Rogers was able to add the total weight of prunings to date, which amounted approximately to between one-sixth and one-seventh of the total weight of trunk and branches. Unfortunately it is not possible to give the complete figure for total weight of prunings on the Bramley's Seedling and Worcester Pearmain trees under review but it is very doubtful whether the total prunings, which very generally consisted in a light thinning of crossing and crowded branches, would amount to one-sixth of the total weight of trunk and branches over the whole period.

With regard to the relationship of root to stem (i.e. trunk and branches) Rogers and Vyvyan found the weights remarkably constant for Lane's Prince Albert on the four different rootstocks when lifted from East Malling soil. "In nearly every case the stems weighed two-and-a-quarter to two-and-a-half times as much as the roots." It is however very doubtful whether it would be safe to expect the same relationship to apply to other scion varieties five years older, even though grubbed from the same soil.

Whilst the method of excavation and grubbing already described ensured that a large proportion of the heavy framework roots were obtained, the circle excavated was of uniform diameter for each variety and bore no relationship to the spread of branches on any particular rootstock. Thus it seems certain that a greater proportion of the framework roots of the smaller trees must have been obtained and weighed than of the most vigorous ones. Before examining the weights of various parts of these trees and comparing the results with those obtained through a measurement of the area of cross section of stem, the general conclusions drawn by Knight and Hoblyn should be recalled (12). Working with the scion variety Grenadier upon the clonal No. VI rootstock, they concluded first, that the proportion of stem was constant throughout a wide range of size of tree and second that the ratio of weight of scion to cross sectional area of main stem increased with the weight of tree.

Tables XIV and XV give the weights of the different parts of these trees and compare their order of vigour as expressed by total weight of tree with that calculated from the area of cross section of stem.

Taking first the total weight of stem (i.e. trunk and branches) Rogers found that Lane's Prince Albert on No. I averaged two-and-a-half times as much and on No. XVI five times as much as on No. IX. In the case of Bramley's Seedling the greatest range of weight is between the trees on No. IX and of OF 5, which latter are approximately four times as heavy. Those on No. I are two-and-a-half times as heavy as those on No. IX. In making this comparison it must be remembered that it has already been reported (9) that all these trees of Bramley's Seedling on No. IX were unfortunately already seriously scion rooted. Only two trees of Worcester Pearmain on No. IX were scion rooted to a very slight extent, and the weights here show the trees on No. XII to be five times as heavy and those on No. I to be twice the weight.

In the case of Rogers' figures where all the roots were carefully excavated the relationship remains approximately the same, but, as Tables XIV and XV show, the extreme differences are not quite as great. This is almost certainly mainly due to the varying proportion of roots removed by the grubbing process. For example, in Table XIV, whilst the proportion of total stem to excavated

TABLE XIV.

Average Weights of Branches, Trunks and Roots in Kilograms of sixteen year old Bramley's Seedling on Various Rootstocks.

Rootstock	IX	VIII	VII	III	X	IV	V	II	I	XV	VI	XI	XIV	XIII	E	F	XII	XVI	OF5
No. of trees	(6)	(3)	(8)	(8)	(8)	(8)	(8)	(7)	(7)	(8)	(8)	(4)	(3)	(8)	(3)	(4)	(8)	(7)	(4)
Branches	77	87	112	116	110	121	151	161	172	162	175	169	191	203	219	216	247	242	268
Trunk	12	11	18	21	19	20	24	28	26	24	26	24	25	26	28	30	34	28	34
Total Stem	89	98	130	137	129	141	175	189	198	186	201	193	216	229	247	246	281	270	302
Roots Excavated	21*	18*	34	28	42	32	40	33	40	57	43	70	58	51	48	66	48	60	62
Total Tree Weight†	110	116	164	165	171	173	215	222	238	243	244	263	274	280	295	312	329	330	364
Order by Weight	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Order by Cross Section	2	1	4	6	3	5	7	13	14	9	12	8	10	11	16	18	19	15	17

* These trees all showed some roots from the scion which were included in above weights.
† Prunings and unexcavated roots not included.

TABLE XV.

Average Weights of Branches, Trunks and Roots in Kilograms of fourteen year old grubbed Worcester Pearmain on Different Rootstocks.

Rootstock	IX	VII	VI	X	I	E	IV	XIII	XIV	V	II	OF5	XV	III	XI	F	XVI	XII
No. of trees	8	8	8	8	8	1	8	8	3	8	8	6	8	8	7	3	8	8
Branches	13	23	26	29	31	38	37	39	39	42	44	45	45	51	46	51	64	71
Trunk	4	7	8	9	8	9	10	10	9	12	14	11	13	12	11	12	13	15
Total Stem	17	30	34	38	39	47	47	49	48	54	58	56	58	63	57	63	77	86
Roots Excavated	4	9	9	10	9	10	10	11	13	13	12	15	15	13	20	15	18	17
Total Tree Weight	21	39	43	48	48	57	57	60	61	67	70	71	73	76	77	78	95	103
Order by Weight	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Order by Cross Section	1	2	3	4	6	5	8	9	7	12	16	10	14	13	11	17	15	18

root for Worcester Pearmain on No. IX is about 4 to 1, in the case of No. I it is nearly 5 to 1, and in that of No. XII it is nearly 6 to 1. However, although the total weight of tree figure sometimes lessens the difference between trees on two rootstocks, there are very few instances in which the actual order is changed.

In comparing the relationship of branches to trunk there appears in both varieties to be a definite tendency for the trees on the more vigorous rootstocks to have a higher proportion of branch weight. Thus whilst the branch weights of Bramley's Seedling on Nos. IX, VII, X and IV are round about six times that of the trunk weight those on Nos. F, XII, XVI and OF 5 are from seven to eight times as heavy.

The actual extreme differences in ratio of branches to trunk are from 5·5 to one for trees on No. III to 8·6 to one for trees on No. XVI. Bearing in mind the gradual increase in these proportions with tree size, there are one or two noteworthy exceptions. In particular, trees on No. II are disproportionately heavy with both varieties, whilst again in both cases trees on No. IX tend to be disproportionately light.

Of the stronger stocks No. XVI in both cases tends to make stems lighter than No. XII in comparison with their branches, while the trunks of Bramley's Seedling on OF 5 and Worcester Pearmain on Nos. V and XV also tend to be disproportionately heavy.

These individual peculiarities are further emphasized when a comparison is made of the actual order of vigour suggested by the two measurements, weight of trunk and branches and cross section of stem. Since the weight per unit volume of the trunk wood appears to be very nearly constant, these disproportionate weights must imply a thicker stem.

Whilst the deductions which would have been drawn from either record are very generally the same, there are several notable exceptions in both Tables. Thus Bramley's Seedling on Nos. XVI and XII differ significantly in their cross sections but not by weight, and the trees on No. II, while still remaining in the same group come considerably lower on the scale by weight than by cross section.

Worcester Pearmain on Nos. II and XVI again do not differ significantly in trunk thickness but, in weight the trees on No. II are significantly the lighter. Again Worcester Pearmain on No. V is significantly larger than on No. XIII in cross section of trunk, but in weight there is very little difference between them. Otherwise the comparison of the trees by either record is remarkably confirmatory.

Thus while Knight's and Hoblyn's conclusion that where all trees are on a single stock and treated alike, the ratio of tree weight to trunk cross section increases with tree size, is generally confirmed, it is evident that a change of rootstock may modify the relationship.

ROOTSTOCK EFFECT UPON CROPPING.

Although there is but one way of measuring the tree's cropping by the count, weight and grading of the fruit produced, there are a number of aspects from which these figures must be considered.

First of all there is the earliness of coming into bearing of any particular variety upon each rootstock. Secondly there is the total crop borne by the tree at any given period in its history. Thirdly there is the relationship between this crop and the size of the tree as expressed by the various measures of vigour already discussed. Presumably there may be a time when the relationship between cropping and growth factors will so interact that the tree comes to a standstill in one or other or both directions. That period as has already been suggested does not appear to have arrived in any of the combinations of stock and scion under observation, and until it has, it is impossible to give any ultimate measure.

(i.) *Precocity or earliness of coming into bearing.*

In the earlier reports already referred to (3, 4 and 5), data were presented giving the number of fruits borne in the first six and seven years and the total crop in lbs. during the first ten years. Whilst the former figures emphasized the outstanding behaviour of all varieties upon No. IX in these early years, the latter showed that, owing to the very fact of size, trees on other rootstocks had in the case of Lane's Prince Albert and Worcester Pearmain actually overtaken these precocious little bushes.

Another six years have passed and a study of the accumulated crop records from year to year will disclose the situation. Up to the end of the ninth year the trees of Lane's Prince Albert (see Appendix, Table (*a*)), on No. IX actually maintained the lead, closely followed by those on Nos. VII, IV, X, XI and I. From the tenth year onwards the trees on No. IV actually head the list, still closely followed by those on No. I and Crab A, which latter actually beat the trees on No. IV in the fifteenth year (1933). In the final year of cropping (1934) the Lane's Prince Albert on No. XVI and Crabs A and C come to the top of the Table, closely followed by those of Nos. IV, I, VI and OF 5. It appears as if the very vigorous trees are beginning to come to " maturity " in their sixteenth year, whilst the most prolific amongst the vigorous ones are still holding their own.

The history of the Bramley's Seedling series is not quite the same (see Appendix, Table (*b*)). The very striking lead taken by the trees on No. IX up to the end of their tenth year (1928) is lost in 1929 and onwards to the trees on No. IV (in 1929 and 1931) and No. II in 1930 and 1932 to the present time. The trees on No. I followed closely, but on the very vigorous stocks only those on OF 5 and XVI began to crop heavily from 1932 onwards.

Almost from the start the crops of the Worcester Pearmains on No. IV equalled those from No. IX (see Appendix, Table (*c*)). By the ninth year the former had established their ascendency only to be excelled a year later (1928) by the trees on No. XI which maintained a remarkable and exceptional record up to the time the trees were grubbed (1932). The trees on No. IV maintained good crops throughout, very closely followed by those on No. I. From the more vigorous stocks trees on OF 5 and No. XVI began about their tenth year (1928) to bear consistently.

A number of factors have contributed to the poor cropping record of all the Cox's Orange Pippin trees, but up to the end of their eleventh year (1931), the trees on No. IX maintained a market supremacy. In 1932, the trees on No. IV took the lead and remained at the head of the list up to the end of 1934, though from 1932 the trees on No. II became serious competitors.

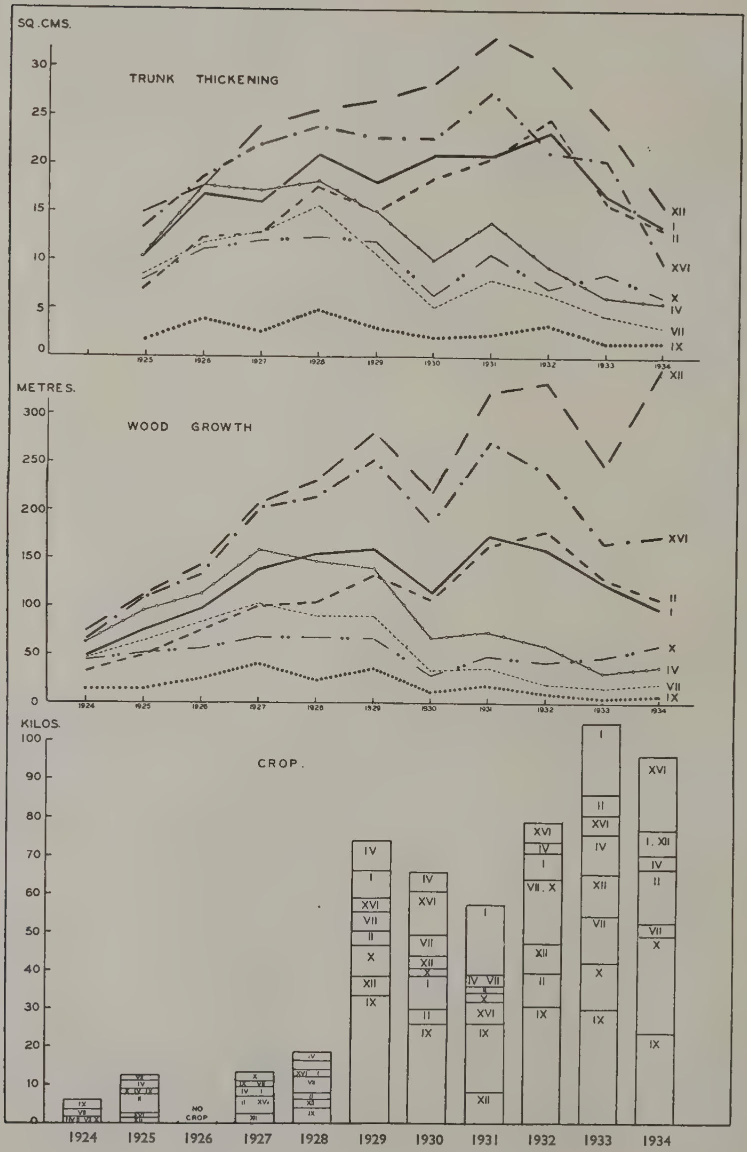
(ii.) *Early Cropping in relation to vigour.*

The continued heavy cropping of all varieties on Nos. IX, IV and VII has undoubtedly had an effect on the vigour of the trees. It is possible to show this relationship between cropping and growth in diagrammatic form (see diagram C) in the case of Lane's Prince Albert on which wood measurements have been maintained to date. In addition, Lane's Prince Albert is a variety which has not so far exhibited marked " on " or " off " bearing years, nor have individual trees of this variety become markedly out of step in this respect, as they have already done in the case of Bramley's Seedling. It will therefore not be misleading to trace the mean growth and cropping reactions for each group of trees of Lane's Prince Albert from year to year.

In diagram C the annual increments in area of cross section of stem are added for comparison with the annual wood growth but it should be recalled that Hoblyn's examination of such data led him to the conclusion that there is an intimate relationship between stem thickening and cropping—a large increment being followed by a heavy crop, and a year of heavy cropping being associated with a small stem increment (13). More recently he has indicated that this relationship may be modified by cultural conditions (7).

Whilst the comparison of the annual wood growths and increments of cross section of stem of Lane's Prince Albert gives evidence of a common trend in both cases from 1925 to 1928, and again from 1930 to 1934, the wood growth appears to fluctuate more violently, and there are certain seasons and individual cases in which the tendency to make wood and increase trunk are in an opposite direction. For instance, compare the effect of the 1929 season upon both factors, or again, compare the wood growth of the trees on No. XII in the season of 1934 both with that on other stocks and with its own increment of cross section of trunk in the same year.

DIAGRAM C.



Annual Trunk thickening, Wood Growth, and Crops of Lane's Prince Albert on 8 Rootstocks (from the 6th to the 16th year).

Prior to 1928 no combination of stock and scion had yielded as much as 20 kilos of fruit per tree, yet the continuous small crops from Nos. IX, IV, VII and X were already tending to slow down their wood growth, though the trees on other stocks were still growing away rapidly. From 1929 to 1934 inclusive, the trees on No. IX cropped regularly in the neighbourhood of some 30 kilos, whilst those on No. IV had five crops approaching 70 kilos per annum. The falling off in wood growth is consistent, but it is interesting to note that in 1931 the trees on No. IV only produced an average of about 40 kilos and in that comparative " off " year show a much greater trunk increment, though no significant increase in wood growth.

The trees on Nos. I and II, more slowly maturing stocks, may next be considered since they fluctuate somewhat together. The wood growth of the trees on No. I was first steadied down by the considerable crop of 67 kilos in 1929, but in 1930 it drops still further when the trees are producing about half that weight of fruit; there is a considerable wood growth increment in 1931 despite an average crop of 56 kilos, but three successive crops of from 70 to 100 kilos from 1932-4 finally slows down the new wood growth. Whilst these three latter years are similarly mirrored in the record for increment of cross section, this measure of vigour is definitely depressed in 1931 but augmented in 1930, which is exactly contrary to the record of new wood growth.

The trees on No. II though not quite so prolific behaved very similarly. The first considerable crop (49 kilos) in 1929 did not affect the wood growth, but it showed clearly in stem increment. On the other hand, despite a smaller crop (30 kilos) wood growth slowed down in 1930 whilst there was a marked stem increment. In 1931-2, the trees on No. II only bore moderate crops of 34 and 40 kilos which did not affect the wood growth or stem increment, but two successively heavy crops in 1933 and 1934 (85 and 65 kilos respectively) depressed both measurements of vigour.

Trees on the very vigorous No. XVI were bearing about 60 kilos of fruit in 1929 and 1930. Whilst it took the two crops to steady down the wood growth both showed their effect upon trunk increment; however a comparatively " off " year (32 kilos) in 1931 was immediately reflected in both measurements. Three further crops in the neighbourhood of 80 to 90 kilos (1932-4) appear to have affected both new wood growth and trunk increment similarly. The most vigorous trees of all—on No. XII—bore two crops of 38 and 48 kilos in 1929 and 1930 at the end of which year the wood growth showed a marked falling off and the trunk increment began to steady down. In 1931 these trees had a definite " off " year from which both new wood growth and stem increment profited. A crop of 47 kilos in 1932 steadied down the wood growth but definitely depressed trunk increment. Both wood growth and stem increment reflected the crop of

65 kilos in 1933 but only the latter measurement responded to the still heavier crop of 77 kilos in 1934.

Although similar diagrams have been prepared showing the crop and stem increments from year to year for Worcester Pearmain and Cox's Orange Pippin, and in biennial periods for Bramley's Seedling, it would appear that the comparisons cannot by any means be pressed too closely owing to the many factors affecting trunk increment. Suffice it to say that the general trend of these diagrams is the same as the one here presented in illustrating that relatively heavy successive crops are ultimately reflected in the vigour of the tree. A consideration of the total crop in relation to the total area of stem and branches will give some idea of what may be called a relatively heavy crop for any particular stock-scion combination. Whilst it only took several crops of 20 kilos to affect the vigour of certain combinations, it has taken successive crops of three to five times that amount to have any serious influence upon others. It would seem then that the weight of fruit in any one year or the number of years over which a tree must crop before the effect is seen upon the tree's vigour varies with each individual combination of stock and scion and also with varietal habit.

(iii.) *Total Crop.*

As will be gathered from the foregoing discussion, the actual total crop per tree at the end of any one year may give a one-sided view of the tree's actual performance. For whilst the trees on certain rootstocks may have been bearing small crops continuously over a period of years, those on others may have been making a framework which in one or more years is capable of bearing as much fruit as a less vigorous tree over a long period.

Even so there are certain very interesting facts to be noted from a comparison of the four varieties upon the range of rootstocks to date.

Table XVI shows the whole range of rootstocks, worked with Lane's Prince Albert, arranged in ascending order according to cropping.

In this case the precocious dwarf trees on Nos. VIII and IX are already at the bottom of the list, whilst trees still in full vigour of growth such as those on Nos. I, VI and even XVI have worked their way to the top. It is interesting to note however that the trees on the once vigorous No. IV—now very low in the order of vigour—still maintain a place amongst this new high cropping group. Between this heavy cropping group, all of which have borne over 400 kilos and which have borne approximately twice as much as the trees at the bottom of the list, is a large group containing a range of trees which have cropped from 315 to 360 kilos.

Table XVII gives the same data for Bramley's Seedling but it is obvious with this variety that the trees on the strongest stocks have not yet reached the same stage of maturity as in the case of Lane's Prince Albert.

TABLE XVI.

Total Crop in Kilograms per tree of Lane's Prince Albert (*Plot* 11) *on different Rootstocks* (*sixteen years old at end of* 1934).

Stock	IX	H	VIII	V	XII	F	X	III	XV	E	XIV	XIII	VII	II	XI	OF5	VI	I	IV	A	XVI	C
No. of trees	(20)	(2)	(8)	(20)	(29)	(4)	(20)	(19)	(18)	(4)	(8)	(20)	(19)	(20)	(9)	(4)	(20)	(20)	(9)	(5)	(20)	(4)
Crop	204	217	220	259	290	315	318	319	321	322	323	350	353	358	361	412	420	439	440	441	443	455
Order by Crop ..	22	21	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
Order by Trunk Area	22	20	21	13	1	9	18	17	10	2	4	8	19	11	16	5	6	7	15	12	3	4

TABLE XVII.

Total Crop in Kilograms per tree of Bramley's Seedling (*Plot* 12) *on different Rootstocks* (*sixteen years old at end of* 1934).

Stock	VIII	IX	V	XII	XIII	XIV	VI	X	E	XV	F	XVI	VII	III	OF5	XI	I	IV	II
No. of trees	(3)	(8)	(8)	(8)	(8)	(3)	(8)	(8)	(3)	(8)	(4)	(8)	(8)	(8)	(4)	(4)	(8)	(8)	(8)
Crop	329	362	373	385	391	400	401	407	407	423	434	447	449	458	458	493	522	552	584
Order by Crop	19	18	17	16	15	14	13	12=	12=	10	9	8	7	6=	6=	4	3	2	1
Order by Trunk Area ..	19	18	13	1	9	10	8	17	4	11	2	5	16	14	3	12	6	15	7

TABLE XVIII.

Total Crop in Kilograms per tree of Worcester Pearmain (*Plot* 13) *on different Rootstocks* (*fourteen years old at end of* 1932).

Stock	E	V	XIII	VII	F	VI	III	XII	II	X	XIV	XVI	IX	XV	OF5	I	IV	XI
Number of Trees	(1)	(8)	(8)	(8)	(3)	(8)	(8)	(8)	(8)	(8)	(3)	(8)	(8)	(8)	(6)	(8)	(8)	(7)
Crop	58	82	90	103	104	105	107	107	113	114	116	121	126	130	141	151	170	194
Order by Crop	18	17	16	15	14	13	12=	12=	10	9	8	7	6	5	4	3	2	1
Order by Trunk Area	14	7	10	17	2	16	6	1	3	15	12=	4	18	5	9	13	12=	8

Whilst the dwarfed trees upon Nos. VIII and IX stocks are again at the bottom of the list, it is generally those on the stocks of intermediate vigour such as Nos. I, II and XI which here head the list with nearly double the crop, though it appears that it will not be long before trees on OF 5 and No. XVI outstrip them. In the case of Bramley's Seedling there is now a very large group of stocks bracketed together for cropping at the bottom of the list—some ten in all—including such obviously incongruous bed-fellows as Nos. IX, V, XII, VI and X, which in actual fact have such totally different cropping histories. Again, a group closely following upon the performance of trees on Nos. II and IV at the moment embraces Nos. XVI, VII and OF 5—in fact, trees of very different habit. It seems certain that trees on these different rootstocks are actually in very different stages of maturity.

The data for the Worcester Pearmain trees—two years younger than the series just dealt with—show, in Table XVIII, the trees on No. IX still to be cropping heavily, though there are two significantly more productive groups containing trees on OF 5 and Nos. I, IV and XI.

In the group at the bottom of the list will be found trees on Nos. V, VI, XII and VII, which latter suffered severely from Apple Canker and this necessitated severe pruning.

Table XIX gives the position for the Cox's Orange Pippin trees at the same age.

TABLE XIX.

Total Crop in Kilograms per tree of Cox's Orange Pippin (Plot 32) *on different Rootstocks (fourteen years old at end of* 1934).

Stock	XV	VI	XVI	III	XIII	X	I	IX	V	II	IV
No. of Trees	8	15	16	16	16	15	16	16	16	16	8
Crop	44	46	53	57	57	65	65	72	72	85	112
Order by Crop	11	10	9	8=	8=	6=	6=	4=	4=	2	1
Order by Trunk Area ..	2	7	1	9	4	8	10	11	6	3	5

The trees on No. IV still stand well away at the head of the list, though the far more vigorous trees on No. II come a good second. The trees on Nos. IX and I come bracketed in a third group, whilst the lowest cropping group include trees on Nos. VI and XVI.

It seems clear that, besides definite scion partialities for particular stocks—of which there are several notable examples in these cropping tables—these different varieties, when compared, are at different stages of maturity even upon the same rootstocks. In order to grasp the situation clearly, it will be as well to concentrate upon ten of the principal rootstocks.

Table XX gives the order of cropping of the four varieties upon these ten rootstocks.

TABLE XX.

Total Crop of Four Varieties on Ten Rootstocks, as a Percentage of the Average for each Variety.

Stock	I	II	IV	V	VI	VII	IX	X	XII	XVI
Lane's Prince Albert	125	102	125	73	119	100	58	90	82	126
Order	(3)	(5)	(2)	(9)	(4)	(6)	(10)	(7)	(8)	(1)
Bramley's Seedling	116	130	123	83	89	100	81	91	86	100
Order	(3)	(1)	(2)	(9)	(7)	(4)	(10)	(6)	(8)	(5)
Worcester Pearmain	127	95	143	69	88	86	106	96	90	102
Order	(2)	(6)	(1)	(10)	(8)	(9)	(3)	(5)	(7)	(4)
Average of three varieties above	**123** (2)	**109** (4)=	**130** (1)	**75** (10)	**99** (5)	**95** (6)	**82** (9)	**92** (7)	**86** (8)	**109** (4)=
Cox's Orange Pippin	91	119	157	101	65		101	91		74
Order	(6)=	(2)	(1)	(4)=	(8)		(4)=	(6)=		(7)

The trees on No. I are very prolific in three out of the four combinations and indeed hold on the average second place, those on No. IV being with all combinations outstandingly productive. The performance of trees on No. II is very good with Bramley's Seedling and Cox's Orange Pippin but up to the present mediocre with Lane's Prince Albert and Worcester Pearmain. Even so this stock comes a good third for cropping. With a single curious exception—that of Cox's Orange Pippin—the trees on No. V have always been in the poorest cropping group, whilst those on No. VI have not been much better except in the case of Lane's Prince Albert.

The position of the trees on No. VII is prejudiced by the outbreak of Canker upon the Worcester Pearmains, but like the trees on No. IX they are now suffering by comparison of tree for tree crops by their true semi-dwarf character. The performance of trees on No. X is as remarkably constant in its mediocrity as it is distinct in habit of growth. Of the two very vigorous rootstocks, it is obvious that the trees on No. XVI are coming into much more early bearing than those on No. XII. Indeed at the moment the average cropping position of trees on No. XVI would be bracketed with those on No. II in this list of ten, whilst those on No. XII would be well at the bottom.

Mere size of tree is by no means the deciding factor in productivity for in nearly all the combinations trees on Nos. II and V, Nos. I and VI, Nos. XII and XVI, and not infrequently Nos. X and IV are respectively similar in vigour. Although they do not appear to be of markedly different maturity, yet, they possess strikingly different crop records to date.

(iv.) *Total Crop to Total Weight of Branches and Stem.*

Perhaps the most striking aspect of rootstock influence is to be found in the comparisons of total weight of fruit with total weight of above ground growth of the various combinations of stock and scion. The eight trees of Lane's Prince Albert which Rogers excavated at ten and eleven years showed that, whilst the trees on No. IX had produced over four times the weight of fruit to stem and branches in that time, those on Nos. I and II had produced from two to two-and-a-half times, and those on No. XVI had only just about equalled growth and cropping.

In the case of the Bramley's Seedling and Worcester Pearmain trees which were grubbed in 1934 and 1932 respectively, it is possible to present this further aspect of the relationship between vigour and cropping in greater detail,though it is only at the end of the tree's natural life that this picture can be complete. However the ratios of total crop per tree to total weight of wood produced given in Table XXI do actually present a very fair and remarkable picture of the trees' performance to date.

TABLE XXI.

Ratio of Total Crop to Weight of Trunk and Branches on Ten Rootstocks.

Bramley's Seedling (16 years).		Worcester Pearmain (14 years).	
Stocks in order of Tree Weight above ground.	Ratio of Crop to Tree Weight.	Stocks in order of Tree Weight above ground.	Ratio of Crop to Tree Weight.
IX	4·5	IX	7·5
X	3·2	VII	3·5
VII	3·4	VI	3·2
IV	3·9	X	3·0
V	2·1	I	3·8
II	3·1	IV	3·6
I	2·6	V	1·6
VI	2·0	II	1·9
XVI	1·7	XVI	1·6
XII	1·4	XII	1·2

The figure for Bramley's Seedling on No. IX in the Table is not nearly so striking as it would have been a few years earlier before these trees became scion rooted and started into vigorous growth, though even now it is impressive.

The most interesting feature of all is to compare the ratios of crop to tree weight on some of the groups which do not differ significantly in the latter respect. Bramley's Seedling on Nos. I, II, V and VI are similar in tree weight but whilst the trees on No. II (true Doucin) have produced three times their weight of fruit in sixteen years, those on No. V (improved Doucin) have produced only twice their weight. Again there is a striking difference in the cropping performance of this variety on Nos. I (2·6) and VI (2·0). The Worcester Pearmain's illustrate the same feature well on rootstocks Nos. IV, II and V, all with the same total tree weight but with crop weights ratios of 3·6, 1·9 and 1·6 respectively. There is a remarkable break in the ratio figures between trees on Nos. IV and V, which is almost as striking in the case of Bramley's Seedling.

From yet another angle these figures go to confirm the indications given in the preceding tables.

(v.) *Fruit Size.*

Whilst the question of rootstock effect on cropping is incomplete without considering the effect on size of fruit, it must be remembered that a number of factors may go towards influencing size, the most important of which may be the number of fruits in proportion to tree weight. However, rootstock influence may be stronger than this consideration. A good instance is found in the comparison of fruit size, in two consecutive years, of Bramley's Seedling on Nos. I and V which produced trees of equal weight. Despite the fact that the crops on No. I were nearly double those on No. V in both years the fruit on No. I is significantly larger as shown in Table XXII.

Table XXII.

Per cent. Fruit of Bramley's Seedling in various size grades on two Rootstocks.

	Per cent. in size grade.						Average No. of Fruits per tree.	Average size figure.	S.E.	Sig. Diff.
	1	2	3	4	5	6				
1929										
On No. I ..	2	9	18	23	44	4	423	2·90	0·099	0·32
On No. V ..	—	3	8	17	53	19	218	2·20		
1930										
On No. I ..	1	5	16	18	44	16	487	2·54	0·087	0·29
On No. V ..	—	1	6	6	43	44	287	1·79		

The "Average Size figure" given in the Table represents the average grade of the variety on a particular stock, except that the grade numbers are reversed, the largest size being designated 6 and the smallest 1.

Similar figures have been examined for the varieties Lane's Prince Albert and Worcester Pearmain. In the former case the average size of fruit from trees on No. I is 3·51 and 4·97 whilst that from trees on No. V is 2·90 and 4·07 for the two years. In the case of Worcester Pearmain the differences are not significant.

Such differences in fruit size, it should be remembered, may vary with the heavy and light bearing years of individual trees and with that age of maturity which has already been referred to as reflecting itself upon the vigour.

In Table XXIII an attempt has been made to show that on the average, over a period of years, there have been very considerable differences in size of fruit calculated from the whole crop of each tree.

TABLE XXIII.

Average Size of Lane's Prince Albert Fruits for Total Crop per Tree, 1921-33.

Stock.	No. of trees.	Mean No. of Fruits in 5 Kilos.	Total No. of Fruits.
XVI	20	32·90	2,200
I	20	33·04	2,459
II	20	33·09	1,768
IV	9	34·09	2,528
VI	12	34·20	2,192
X	20	34·77	1,870
XII	29	35·77	1,518
IX	20	36·30	1,305
VII	19	37·36	2,232
V	20	38·32	1,578

Column 3 gives the mean number of fruits in 5 kilograms over the whole period, whilst the total number of fruits given in Column 4 serves to show that this has not been the sole factor in governing the size. For instance, note the good sized fruit of Lane's Prince Albert on No. IV rootstock despite the large number of fruits borne. This applied almost equally to the trees on No. I. Again, whilst the fruits from Nos. V and VII are the smallest over the period, it should be realized that those on No. VII are undoubtedly small as a result of the large number of fruits borne on a small area, whilst those on No. V appear to be inherently small despite the comparatively poor crops borne on much larger trees. Finally it is interesting to compare the trees on the two very vigorous stocks—Nos. XII and XVI. Although the trees on No. XII have borne considerably less fruit they are markedly smaller.

The variety Lane's Prince Albert has been chosen to illustrate this general point because between the years 1921-33 no artificial thinning methods were practised. If such methods were applied intelligently to different stock-scion combinations undoubtedly fruit size in many cases would have been much improved.

When the records are studied from year to year it is of course found that the results vary considerably. A similar comparison of fruit size from year to year from the trees of Lane's Prince Albert on rootstocks Nos. I and IX illustrates the point very well. This is shown in Table XXIV.

TABLE XXIV.

Number of Fruits to 5 Kilograms Lane's Prince Albert, 1922-33.

Stock	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933
No. I	—	32·2	33·1	30·4	43·2	46·3	29·5	38·4	30·0	32·0	28·1	34·4
	—	(0·2)	(1·4)	(8·9)	(0·3)	(9·7)	(13·5)	(66·1)	(38·7)	(56·9)	(70·7	(104·8)
No. IX	34·4	29·3	39·1	27·9	36·7	41·6	27·1	42·8	31·0	34·5	34·4	39·0
	*(0·2)	(1·5)	(6·3)	(9·2)	(0·9)	(11·0)	(4·1)	(33·7)	(26·4)	(26·2)	(30·7)	(29·8)

* The figures in brackets represent the total crop for year in kilos.

TABLE XXV.

Number of Fruits to 5 Kilograms Bramley's Seedling (Plot 12), 1922-33.

Stock	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933
No. I	33·7	24·9	42·7	29·8	31·7	33·1	38·0	42·9	34·7	37·8	31·6	37·9
	(0·4)	(0·8)	(2·5)	(9·5)	(3·6)	(3·6)	(44·0)	(93·0)	(62·6)	(75·3)	(53·1)	(61·7)
No. IX	34·4	22·3	38·0	22·5	27·7	25·4	33·3	33·6	32·2	29·2	32·0	28·0
	(1·1)	(1·0)	(8·5)	(7·3)	(13·2)	(5·4)	(55·8)	(63·5)	(64·4)	(16·3)	(41·7)	(5·9)

TABLE XXVI.

Number of Fruits to 5 Kilograms Worcester Pearmain (Plot 13), 1922-32.

Stock	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932
No. I	—	49·4	64·5	48·7	55·1	50·9	55·2	54·9	55·3	56·9	53·4
	—	(0·2)	(1·6)	(3·3)	(0·3)	(15·7)	(15·8)	(31·0)	(23·4)	(35·1)	(24·3)
No. IX	50·5	51·1	58·6	47·3	43·2	49·8	48·1	No Count	47·1	55·0	54·0
	(0·1)	(0·5)	(2·0)	(4·3)	(0·7)	(10·9)	(10·1)	(28·1)	(15·0)	(31·0)	(23·5)

In the first place there is a very remarkable variation in size from season to season on each individual stock, and this is not always connected with total weight of crop as indicated by the figures in brackets. For instance, note the seasonal influences on size in 1925, 1926 and 1927.

Generally speaking the trees on No. IX have larger fruits than those on No. I between 1922 and 1928, after which period they are smaller. In 1925 and 1927 trees on the two stocks had very similar crops, though a crop of 10 kilos was actually relatively a much heavier one for the trees on No. IX. Yet in both these years there were fewer fruits needed for 5 kilos from the trees on No. IX.

Although after 1928 the crops off No. IX are only about a half those harvested off No. I, the size of the fruit in the former case consistently falls off. It is obvious from the foregoing tables that, relatively to size these trees were cropping much more heavily.

Similar data for the Bramley's Seedling and Worcester Pearmain plantings under review are available, and since they illustrate rather well the tendency to a differential varietal response they are presented here in Tables XXV and XXVI.

In the case of Bramley's Seedling, the fruits from No. IX are larger than those on No. I almost throughout the period. Whilst this may be varietal, it must be remembered that the renewed vigour imparted to the trees on No. IX as a result of scion rooting may be responsible in no small degree for the maintenance of fruit size on that series of trees. As Table XXVI shows although the fruits from trees of Worcester Pearmain on No. IX were generally larger—sometimes very markedly so in the early years—they were approximating to the size of those No. I before they were grubbed. They had also been cropping relatively much more heavily as the mean crop weights show.

A similar comparison of the records of Cox's Orange Pippin shows that during their ten year cropping period (1925-34) the fruits from trees on No. IX have been markedly larger in all years than those on No. I.

OTHER MANIFESTATIONS OF ROOTSTOCK EFFECT ON APPLE SCIONS.

During the past seventeen years many other observations have been made upon the material at East Malling which was raised, planned and planted for these studies. Whilst space does not permit of dealing in detail with these results here, some of the most important must be mentioned since they are so closely connected with the manifestations of vigour and productiveness already analysed in this paper.

In an intensive study of every undamaged leaf on two nine-year-old trees of Laxton's Superb on Nos. V and IX rootstocks, Vyvyan and Evans (14) showed that whilst there was a greater total area on the tree on No. V, the total leaf area per unit length of stem was less than on No. IX. This greater total leaf area on No. V was due to several factors including a larger total number of growths, the smaller proportion of small-leaved bearing spurs, and the mean size of leaf being greater in most categories of growths. Still more recently Vyvyan (11) in developing the method of recording the different types or categories of growths upon an apple tree has demonstrated the remarkable differences in the number of bearing and non-bearing primary and secondary growths on four- and five-year-old Cox's Orange Pippin trees on Nos. I, V, IX and XII. He also showed that most of the growths on No. IX became inactive during June and July instead of before June as in the case of the other three rootstocks. The proportion which continued to grow after July was slightly less and was in accordance with the observations made by Swarbrick under very different conditions at Long Ashton (15).

These characteristic types of growth and their seasonal development naturally affect the amount and position of fruit bud formation so frequently referred to in the earlier papers (3, 4 and 5) by the present writer. As early as 1923 it was pointed out that a very different percentage of this blossom set when the same variety was worked upon different rootstocks (16). Much more recently Hoblyn and Bane (7) found that the same phenomenon accounted in no small degree for the poor cropping of trees on No. V rootstock. That in many seasons there was a noticeable difference in the time of blossoming of a single variety on the series of rootstocks was shown in a paper by Grubb and the present writer (17). On the average of three years the flowering of Lane's Prince Albert varied to the extent of seven days in the first opening of the blossom and four days in full blossoming. In the season of 1927 when a severe frost occurred on April 26th, 27th and 30th, this variation was sufficient to be very important economically.

The effect of rootstock upon fruit colour was reported in 1923 (16) and a method for accurately recording amount of colour was tried out by Rogers (18) for two years mainly on Bramley's Seedling and Lane's Prince Albert on rootstocks Nos. I, II, VII, IX, XII and XIII. The crops from No. IX rootstock have usually given the best coloured fruits, those off trees of No. II being inferior in this respect to those from Nos. I and VII. More recently intensity as well as amount of colour has been taken into consideration by Hoblyn and Bane (7) and has been shown to be influenced by rootstock as well as manurial treatments.

A number of trials with apples from different rootstocks kept in ordinary and cold storage conditions have been made with a view to testing the possible influence of the stock. Little information has as yet been published though in one experiment described by Kidd and West (19) it was clear that marked differences were present in the particular season both in the case of low temperature breakdown in Bramley's Seedling and of fungal rotting following senescence in the case of Lane's Prince Albert. It is pointed out that these results—

which incidentally showed that apples from trees on No. IX do not necessarily keep worse than those from trees on other stocks—may have been affected by the varying stages of maturity of the apples from particular rootstocks and that this must be very carefully considered in judging storage quality. Fruits from trees on No. IX are usually considered to ripen earlier than on most other stocks and probably merely require to be picked earlier to ensure satisfactory storage.

In examining the chemical composition of Bramley's Seedling and Worcester Pearmain apples from trees on four rootstocks, it is interesting to note that Archbold (20) reported marked differences in the fruits from No. V rootstock compared with those from Nos. IV, VI and X.

Horne has been working since 1930 on the resistance of fruit to storage rots and he included the effect of rootstock in his investigations. Although stock influence in this respect may vary with different varieties, seasons and cultural or manurial conditions, significant differences have been reported (21) during the past four years. For instance, in 1930 and 1931 Bramley's Seedling from Nos. IV and VI produced more resistant apples than from rootstocks Nos. V and X.

In 1930 Wallace (22) gave examples of significant differences in storage quality due to rootstocks under certain environmental conditions. For in the 1929 season Worcester Pearmain apples from trees on No. IX rootstock showed much more internal breakdown than the fruits from Nos. I, II, V and XII. Whilst in the case of Bramley's Seedling, the fruits from trees on No. I exhibited much more core-flush and internal breakdown than those from fruits off No. V. Significant effects on the chemical composition of apples due to rootstock were also reported. Apples grown on No. IX stock were said invariably to contain higher percentages of sugars than comparable fruits from any of the other stocks tested. Fruits from Bristol OF 5 invariably produced fruits of high potassium content. The question of fruit composition in relation to the rootstocks has been more recently elaborated by Warne and Wallace (23) from a series of trees raised in the East Malling nurseries and planted out at Long Ashton at the same time as the main experimental plots under discussion.

Moore (24, 25 and 26) has advanced considerable evidence over a number of years to show that the incidence of Apple Scab (*Venturia inaequalis*) on the variety Cox's Orange Pippin has been influenced to a considerable degree by the rootstocks on which the trees were worked. Results were seen both in leaf- and fruit-estimations of infections. Although seasonal variations in this influence have been evident, trees on Nos. I, II, V and IX commonly showed more infection than those on Nos. XIII and XV. In the case of Powdery Mildew (*Podosphaera leucotricha*) these two rootstocks again appear to have induced resistance whilst trees on No. I were outstandingly susceptible to infection.

In a single year's survey made on Bramley's Seedling in 1928, the trees on No. X were, for their size, more heavily infected than any others. Trees on No. I again showed considerable infection.

Although there have been outbreaks of Apple Canker (*Nectria ditissima*) especially upon some of the Worcester Pearmain and Cox's Orange Pippin trees no satisfactory method of recording incidence accurately has as yet been evolved ; the age of the canker forming a serious obstacle. The fewest number of cankered Worcester Pearmains were found on Nos. I and V, whilst the most serious outbreaks have been on Nos. VII and IV. The trees on the very vigorous stocks—especially No. XII showed considerable susceptibility, and this has been strikingly confirmed on Cox's Orange Pippin trees, when observations were made on two demonstration plots in Surrey and Hampshire.

On the other hand whilst these rootstocks have shown different degrees of resistance to Woolly Aphis—and the variety Northern Spy has shown immunity—when used as rootstocks, Le Pelley (27) showed that they do not in any way appear to pass on this degree of resistance or immunity to the scion. Again, Wormald and Grubb (28) proved that whilst different rootstocks possessed different degrees of susceptibility to Crown Gall disease (*Bacterium tumefaciens*), different scion varieties tended to make the stocks more or less susceptible.

In the case of the physiological trouble now commonly known as Leaf Scorch, Grubb and the present writer (29) first drew attention to the fact that trees on certain rootstocks of the Doucin types—especially those on No. V—and on some seedlings, were particularly prone to this disease. Wallace's (30) identification of this symptom with potash deficiency at once suggested the possibility of a differential response of a single variety upon different rootstocks to manurial treatments. A considerable body of evidence has now been published (7, 23 and 31) which proves that this is the case. The poor performance of trees on No. V rootstock even under conditions of good cultivation is still outstanding though the effect of grass in improving both cropping and quality of fruit off this stock has been noted (7).

There remains a considerable amount of data, as yet unpublished, which sheds further light on many of these aspects and which will be dealt with in due course.

Attempts to Elucidate Causes and Mechanism of Rootstock Effect.

Although this is not the place to discuss the theories which have been put forward to explain rootstock effect, the picture would be incomplete if mention were not made of the principal lines of investigation, which have been followed up on this experimental material.

The long established tradition that rootstock effect depended upon the depth and size of the root system has definitely been disproved, first by the excavation and root analysis work of Rogers and Vyvyan (8 and 32) and then by the conception of a constant stem/root ratio for any given scion/stock combination on a given soil—for which there has accumulated very considerable evidence from a number of experiments.

It will be remembered that the detailed excavation of ten-year-old trees on the vigorous No. I and dwarfing No. IX revealed that in actual fact the roots of the dwarf trees on the latter stock had penetrated nearly 10 feet down whilst those of the vigorous trees had not descended so deeply—partly due to the limit set by the layer of sandy " Hassock ". The ratio of stem to root was about the same on both stocks. When these investigations were extended to other soils (8), it was found that the rootstocks adapted themselves to the varying conditions of moisture, aeration and plant food both as regards depth and spread of rooting and also ratio of root to top, though this ratio was again found to be fairly constant.

Knight and Hoblyn's (33, 12) and Vyvyan's (34) studies on younger trees advanced a considerable amount of further evidence for this conception of a constant root/shoot ratio so that the old idea that a tree was dwarfed because it was depending on a " small rootsystem " no longer holds. However after studying the relationship between root, stem and leaf growth, Vyvyan (34) has suggested a mechanism for some aspects of stock/scion effect on vigour. " A constant stem/root ratio " he writes " means that stem and root are both growing at the same rate, e.g. both must take the same time to double in weight. If a vigorous or fast growing variety is combined with a dwarf or slow growing one as scion and stock in a single tree, they must adjust their rates of growth to a common value, thus scion and stock may be expected mutually to affect one another's ratios of growth in a manner similar to that actually observed." In addition, the findings of Vyvyan (14, 11) already referred to both in relation to the leaf areas and also the categories of growths which distinguish the same variety on different rootstocks, suggest a mechanism through which rootstocks are exerting their influence, even though these manifestations may be the effect rather than the cause. Again, Swarbrick (15) found that " individual varieties growing upon different rootstocks have distinct growth curves and that the elongation of growth of trees worked upon No. IX rootstock—which induces precocity of flower bud formation—showed a much more rapid and in some cases an earlier falling off in the growth rate of selected leader shoots ".

From the initiation of these experiments Amos and the present writer (1, 35) kept the morphology of the various root systems in view in the hope that the very obvious differences in number and stoutness of coarse roots, and quantity and distribution of fibre, and general direction of scaffold roots might afford the key to rootstock influence. The later intensified root studies by Vyvyan

(37) have provided ample evidence that these apparent differences in morphological characters are real and are not materially influenced by the nature of the variety used as scion, though the total and relative quantity of root may be considerably influenced. But even if the English varieties of scion, used at East Malling, had been found to alter the morphological appearance of the root system as Roberts and Swarbrick (36) have found certain other scion varieties, mainly American, apparently do, it would not necessarily have followed that the character of the stock's influence would have been altered also.

An examination of the developed root systems in the light of the known behaviour of fourteen- and sixteen-year-old Worcester Pearmain and Bramley's Seedling trees led Bane, Beard and de Wet (9) to the conclusion that apart from the gradual increase in the total number of roots in passing from the most dwarfing to the most vigorous "there appears to be no correlation between type of root system and tree performance". Despite this, it is suggestive that there is a marked similarity between the morphology of the developed root systems of the early vigorous yet very productive stocks Nos. IV and VII, and of the True and Improved Doucins Nos. II and V—the "leaf scorching" stocks so aptly described by Warne and Wallace as "probably relatively poor 'potash' feeders". The fact that trees of a single variety can grow within 15 feet of one another yet exhibit a different response to the same manuring inevitably concentrates attention on the absorbing capacity of the various root systems. It is hoped that a study of this aspect together with the information already coming to hand as a result of intensive observations on the development and nature of the growth of various root systems in the field (38) and of their anatomy in the laboratory (39) may both add to the existing knowledge of the physiology of an apple tree and also possibly offer a practical line for the selection and identification of new rootstocks with desirable characteristics.

It has however already been shown that at least some portion of rootstock influence resides in the stem portion of the rootstock, though Knight's (40, 41) series of double grafting experiments with vegetatively raised stocks at East Malling especially designed to demonstrate the respective parts played by the stem and root portions of the stock led him to state emphatically that the root system is more important than the stock stem in determining scion performance.

Two series of experiments in which the scion was grafted both in the normal fashion upon the stock stem and also direct upon pieces of root, especially designed to elucidate the parts played by the stock stem and root, led the present writer to conclude (42) that clonal vegetative piece roots were at least as potent in imparting characteristic stock influence as when a portion of stock stem was interposed between root and scion. A third series of trees in the plantation now in their fifth year are confirming these preliminary results (43).

Grubb (44) in his double working experiments with apples has shown that an " intermediate " stem betwixt rootstock and ultimate scion does produce measurable effects, though they have never been very striking.

The range of apple rootstocks exhibiting such striking effects when in combination with the scion have been subjected to physiological examination, with the ultimate object of correlating the characteristics of the stocks with definite series of physiological properties. After several years work which tended to emphasize the remarkable likeness between the physiological behaviour of stocks even as widely differing as Nos. I and IX, Knight (45, 46) ultimately reported that although small differences had been found in the transpiration rates of leaves of different apple stocks, these differences were not discernible in the varieties worked thereon. Hereafter Knight developed his investigations into the causes of stock effect along more pomological lines.

After a very brief visit to East Malling in 1932 Hafekost (47) published the results of some tests of the " wilting limit " and " loss of water " to which he had subjected the different apple rootstocks. Whilst it is true that his values for " wilting limit " and " intensity of transpiration " coincided very generally with the known vigour of the rootstocks, once again there was a failure to get confirmatory results when similar tests were applied to the scions budded thereon.

Roach initiated experiments to determine how far rootstock effect might be expressed in terms of the selective action of the roots, etc., in absorbing elements from the soil and supplying them as raw materials to the scion. In 1931 he reported (48) on the spectrographic examination which had been made of the ashes of various portions of Lane's Prince Albert trees on rootstocks Nos. IX and XII. Twenty-one elements were found to occur, but one—molybdenum—occurred only in the wood of the roots and the rootstocks of the six trees on No. IX rootstock. It was absent from all parts of the trees on No. XII. These preliminary results, which are being followed up by an attempt to make quantitative analyses, suggest that " through its roots a rootstock may exert selective action in absorbing elements from the soil, and that one rootstock may differ from another in the way it exerts this selective action ".

Warne and Wallace (23) have made an investigation of the possible relationships between rootstock effect and chemical factors. Since an examination of trees on certain rootstocks prone to leaf scorch had shown lower amounts of potash in the organs of the trees on Nos. II and V, this seemed a promising line. Whilst as already mentioned, many interesting and significant chemical differences were demonstrated, no chemical explanation was forthcoming for the characteristic dwarfing effect of No. IX and there was only complete agreement in one feature—a high $\frac{K}{N}$ ratio in shoot composition of the trees on the very vigorous rootstocks Nos. XII and OF 5. These workers conclude by stating that " many of the outstanding rootstock effects could not be explained on the basis of the chemical characters examined ".

DISCUSSION.

In the early stages of these investigations it was impossible to predict to what extent rootstock effect upon the growth and cropping would persist or whether the trees on these different vegetative stocks would " even up " in the course of years. The evidence presented in this paper no longer leaves any doubt that rootstock influence does endure and even becomes accentuated. The simple relationships between vigour and cropping exhibited early on in the experiments upon such rootstocks as Nos. IX and I have now been supplemented by the more complex behaviour of trees upon Nos. IV and VII. It has become apparent that even now—after sixteen years—the trees on certain rootstocks such as Nos. XVI and XII have not reached that balance between growth and cropping, which has been called " maturity". As yet the trees on the most dwarfing and precocious rootstocks show no signs of deterioration though it was predicted that they would be short lived.

Whilst it is impossible as yet to say what will be the ultimate fate and full record of the trees on any particular rootstock, it is obvious that the poor cropping record of all varieties upon the No. V rootstock and the outstanding performance of the trees upon No. IV have already been very marked, and, on a large scale over a period of years must be of great economic importance to the fruit grower. To the research worker the distinctive behaviour of a single variety on these two varieties of Doucin has opened up a new vista of possibilities.

Though these general tendencies are remarkably consistent with the four scion varieties included in these investigations, there are equally striking individual scion partialities in combinations with such rootstocks as Nos. I and II.

In view of the data assembled here it seems hardly necessary to emphasize the fact that relative vigour is only one amongst the many important manifestations of rootstock effect. Far more striking and far more difficult to explain is the relationship of vigour to productivity, trees of the same size on different rootstocks producing very different weight and quality of fruit. It might be thought that the phenomenon of two trees of the same variety growing side by side on different rootstocks yet manifesting obviously different responses to a common manurial treatment would clearly demonstrate that rootstock effect was purely a problem of nutrition. Up to the present however it has been no easy task even by the adaptation of special manurial and cultural conditions to bring the trees of relatively poor performance on No. V up to the standard of their productive neighbours. As yet there are no signs that any known manurial treatment will so radically alter the performance of trees on such stocks as Nos. IX, IV, VII or XII that they can be brought to resemble one another in habit or performance.

Although, as has been shown, a considerable body of very careful work has been applied to a solution of this problem, the evidence at present seems to suggest that rootstock influence may be due to a complex of factors. The effect is probably produced partly by the stem, partly by the root portion of the rootstock. The growth rate of the stock in relation to the scion and the capacity of particular root systems for absorbing the necessary raw material may both be of importance. That there are too reciprocal, if minor, reactions of the scion even when budded upon the stem of a vegetative stock must not be forgotten. The part played by the actual union between stock and scion also remains to be fully investigated. In a few years the comparative behaviour of varieties growing upon their own roots and worked upon various rootstocks which is now being made should throw light upon this point, though there are already indications that the answer will not be a simple one. There have been manifestations even with apples that certain combinations of scion and rootstock are "uncongenial" or "incompatible" and investigations are already under way to elucidate the causes, since these extreme cases may shed light upon the reciprocal action of stock and scion.

It will readily be appreciated that the successful outcome of such researches would not only add to the general knowledge of the physiology of the apple tree and so place routine cultural operations on a much more intelligent basis, but would also render possible the choice of a new and better race of rootstocks selected, not empirically as heretofore, but in the confident expectation of certain assured results.

Application of the Results.

The present writer with some of his colleagues has explored the best methods of vegetative production of fruit tree rootstocks (52) and the former has pointed out (49, 50, 51) the important bearing this new light upon the many symptoms of rootstock influence must have on investigations with fruit plants. It makes possible a standardization of performance of the horticultural experimenter's material hitherto undreamt of. Many sources of variation can now be traced, explained and avoided, and the layout of horticultural plots can be much more economically planned. The discredit which until recently had so generally fallen upon Field Experiments with Fruit Trees owing to their variable behaviour has now been largely dispelled.

The practical grower and nurseryman have been kept informed through a series of bulletins (52—60) of the new facts which, as they came to light, dispelled the tradition which held the field, up to a few years back, that there were two types of apple tree—the one grafted upon Seedling Crab or Free Stock which was deep rooted and vigorous, and the other worked upon vegetatively raised Paradise apple stocks which were surface rooting and dwarf.

The possibilities of obtaining a more complete measure of success in the nursery and subsequently of utilizing the different types of tree for the most economical planning of the plantation have been suggested from time to time as the investigations developed. There is therefore no necessity to stress these points further here, though doubtless as the story continues to unfold there will be fresh facts to add to the investigator's knowledge of the apple tree and to guide the grower more accurately in his plantings.

The figures presented in this paper have recently been summarized for the fruit grower (60), but it may be as well to draw attention to the most outstanding facts once again.

Trees on the Jaune de Metz Paradise No. IX have continued to crop heavily and maintain healthy growth throughout the period, and though according to total crop per tree they are now left far behind in productivity, a crop reckoning on an acreage basis at suitable planting distances would still tell a very different story.

The big gap between these dwarf trees (on No. IX) and the generally vigorous ones on Nos. I and II has now been filled by two very attractive types of real semi-dwarf trees on No. VII and IV (an un-named selection and the Holstein Doucin). Unfortunately these two stocks which produce quick growing highly productive trees, possess serious disadvantages both in the nursery and plantation. They have therefore never received an unqualified recommendation, and even now should only be tested with great caution.

The really vigorous nature of the supposed "semi-dwarf" Doucin varieties (Nos. II and V), with most scion combinations, has now become apparent. The great inferiority of the so-called Improved Doucin, No. V, in quantity and quality of fruit, has been conclusively demonstrated. The originally vigorous stocks represented by No. I and its inferior No. VI, the Broadleaf and Nonsuch Paradise, have been caught up and sometimes surpassed by the true Doucin, No. II; though all these four rootstocks (Nos. I, II, V and VI) have definitely been shown to exert a different influence upon different scion varieties. In the light of the evidence here presented Nos. V and VI could with advantage be dispensed with altogether. The choice between Nos. I and II would seem to rest mainly with the scion variety. With two varieties the trees on No. I have so far cropped outstandingly better, in the other two the trees on No. II are now definitely outstripping them.

Few of the trees on the very vigorous rootstocks have as yet—after sixteen years—come into full bearing (if this be judged by relation of weight of crop to size of tree) though those of Bristol OF5 and No. XVI (and Crabs A and C) have been the most productive under the soil and climatic conditions at East Malling, No. XII is still outstanding for its vigour and tardy cropping.

SUMMARY.

1. Progress reports of 1920, 1927 and 1929 are summarized.

2. The origin, propagation and method of working the rootstocks and of planning, planting, pruning and treating the young trees uniformly are described. Any possible effects of subsequent cultural and manurial treatments upon the maturing plantation are discussed.

3. Scion rooting of Bramley's Seedling on Nos. VIII, IX, and, to a lesser extent, on Nos. II and VII is recorded.

4. The effects of rootstock upon tree growth as manifested in total wood measurements, weights of sample prunings, thickness of trunk, height and spread of branches and total weight of tree and their inter-relationship are compared, and their relative value discussed.

5. Changes in the relative order of vigour as expressed by total wood growth are reported and the advisability of adhering to a method of grouping rootstocks according to vigour is discussed.

6. Measurement of cross section of stem is used to demonstrate the fact that the vigour of different scion varieties on the same rootstock is affected to a different degree, and that the terms " dwarfing " and " vigorous " are therefore relative only.

7. Whilst the majority of rootstocks—Nos. VIII, IX, IV, VII, X, XII, XVI—appear to exert relatively the same influence upon all four scion varieties, definite scion partialities are now evident upon rootstocks Nos. I, II, V and VI, as shown by vigour and to some extent by cropping.

8. Influence of rootstock on habit of growth of the scion is illustrated.

9. Total weights of branches, trunk and part roots of 246 fourteen- and sixteen-year-old trees are given. Scions on very vigorous rootstocks are found to be four and five times as heavy as those on very dwarfing ones. The ratio of branches to trunk varies upon different stocks. It is shown that different rootstocks modify the ratio of tree weight to cross section of trunk for a single scion variety.

10. Effect of rootstock upon earliness of coming into bearing is emphasized by the outstanding behaviour of trees on Nos. IX and IV, though the former have now been outstripped as the trees on the best of the vigorous (Nos. I and II) and very vigorous stocks (Nos. OF5, XVI and Crab A) reach " maturity ".

11. An attempt to trace the effect of early cropping upon ultimate vigour is made by a comparison of additional wood growth, stem increment and weight of crop from year to year.

12. Total crop per tree at any one period is shown to obscure the real cropping history of particular combinations, though at the end of sixteen years trees on some rootstocks have borne from twice to three times the weight of fruit harvested from others. Scion preferences are evident. A fair comparison, however, is found possible between the average total crops for the four varieties upon certain rootstocks giving trees of approximately the same size and maturity. Whilst trees on No. I appear second on the list those on No. VI are fifth. Again, those on No. II (Doucin) are fourth, those on No. V (Improved Doucin) tenth and last.

13. Total crop in relation to total branch and stem weight give a most striking demonstration of rootstock influence. On No. IX rootstock trees of one variety have produced 7·5 times their own weight in fruit, whilst on No. XII tree and fruit weight are approximately equal at the end of fourteen years. Ratio of crop to tree weight is invariably low for trees on No. V rootstock.

14. Instances are given where rootstock influence upon fruit size outweighs the number of fruits borne in proportion to tree size.

15. The evidence of rootstock effect on leaf areas, growth types, fruit bud formation and position, percentage of blossom setting, time of blossoming, fruit colour, storage quality and resistance to rots, chemical composition of fruit, disease resistance and susceptibility and physiological troubles is briefly summarized.

16. Various attempts to elucidate the causes and mechanism of rootstock effect are reviewed.

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shared in taking these records can sufficiently appreciate the arduous task which has been so admirably and willingly undertaken.

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REFERENCES.

(1) *Hatton, R. G.* Paradise Apple Stocks. Journ. Roy. Hort. Soc., 1917, **42**, 361.

(2) ———. Paradise Apple Stocks. Their Fruit and Blossom Described. Journ. Roy. Hort. Soc., 1919, **44**, 89.

(3) ———. Results of Researches on Fruit Tree Stocks at East Malling. Journ. Pom. & Hort. Sci., 1920, **2**, 1.

(4) ———. The Influence of Different Rootstocks upon the Vigour and Productivity of the Variety Budded or Grafted Thereon. Journ. Pom. & Hort. Sci., 1927, **6**, 1.

(5) ———. The Relationship Between Scion and Rootstock with Special Reference to the Tree Fruits. Journ. Roy. Hort. Soc., 1930, **55**, 169.

(6) *Pearl, R. T.* Apple Rootstocks I—XVI. Journ. of S.E.A. Coll., Wye, 1932, No. 30.

(7) *Hoblyn, T. N.*, and *Bane, W. A.* Apple Manurial Trials. East Malling Res. Sta. Ann. Rpt. for 1933 (1934), 59.

(8) *Rogers, W. S.* and *Vyvyan, M. C.* Root Studies—V. Rootstock and Soil Effect on Apple Root Systems. Journ. Pom. & Hort. Sci., 1934, **12**, 110.

(9) *Bane, W. A., Beard, F. H.* and *de Wet, A. F.* A Comparison of the Root systems of Mature Trees of Bramley's Seedling and Worcester Pearmain on Various Rootstocks. East Malling Res. Sta. Ann. Rpt. for 1934 (1935), 90.

(10) *Beakbane, A. B.* and *de Wet, A. F.* The Root Systems of some Bramley's Seedling Apple Trees. East Malling Res. Sta. Ann. Rpt. for 1934 (1935), 87.

(11) *Vyvyan, M. C.* Leaf Relations of Fruit Trees—II. The Census Method for Recording Summer Growth with Special Reference to the Apple. Journ. Pom. & Hort. Sci., 1935, **13**, 202.

(12) *Knight, R. C.* and *Hoblyn, T. N.* The Effect of Size of Tree on the Relations between Various Records of Roots and Stems of Apples. East Malling Res. Sta. Ann. Rpt. for 1933 (1934), 117.

(13) *Hoblyn, T. N.* Field Experiments in Horticulture. Imp. Bur. Frt. Prod. Tech. Comm., No. 2, 1931.

(14) *Vyvyan, M. C.* and *Evans, H.* The Leaf Relations of Fruit Trees.—I. A Morphological Analysis of the Distribution of Leaf Surface on two nine-year-old Apple Trees (Laxton's Superb). Journ. Pom. & Hort. Sci., 1932, **10**, 228.

(15) *Swarbrick, T.* The Seasonal Elongation Growth of Apple Varieties on Some Vegetative Rootstocks and its Possible Relation to Fruit Bud Formation. Journ. Pom. & Hort. Sci., 1928, **7**, 100.

(16) *Hatton, R. G.* The Influence of Rootstock upon the Tree Fruits. Proc. of 7th International Hort. Congress, Amsterdam, 1923, 94-112.

(17) *Hatton, R. G.* and *Grubb, N. H.* Some Factors Influencing the Period of Blossoming of Apples and Plums. East Malling Res. Sta. Ann. Rpt. for 1924 (1925), 81.

(18) *Rogers, W. S.* Rootstock Effect on Colour and Size of Apples. East Malling Res. Sta. Ann. Rpt. for 1925 (II Supplement) (1927), 16.

(19) *Kidd, F.* and *West, C.* The Effect of Rootstock on the Keeping Quality of Bramley's Seedling Apples. Rpt. of the Dir. of the Food Investigation Board for the year 1933, Part III, Ditton Laboratory, Section A—Fruit and Vegetables.

(20) *Archbold, H. K.* and *Widdowson, E. M.* Chemical Work on Fruit. Rpt. of the Dir. of the Food Investigation Board, 1931, Part IV, Section B., 260.

(21) *Horne, A. S.* Biological work on Fruit. Rpt. of the Dir. of the Food Investigation Board, 1931, Part IV, Section B., 272.

———. Rpt. of the Dir. of the Food Investigation Board, 1932, Part IV, Section B., 282.

———. Rpt. of the Dir. of the Food Investigation Board, 1933, Part IV, Section B., 228.

———. Rpt. of the Dir. of the Food Investigation Board, 1934, Section V, 165.

(22) *Wallace, T.* Factors Influencing the Storage Qualities of Fruits. Proceedings of the First Imperial Hort. Conference, Part III, 1931, 9.

(23) *Warne, L. G. G.*, and *Wallace, T.* The Composition of the Terminal Shoots and Fruits of Two Varieties of Apple in Relation to Rootstock Effects. Journ. Pom. & Hort. Sci., 1935, **13**, 1.

(24) *Moore, M. H.* The Incidence and Control of Apple Scab and Apple Mildew at East Malling. Journ. Pom. & Hort. Sci., 1930, **8**, 229-47 and 283-304.

(25) ———. Further Studies on the Incidence and Control of Apple Scab (*Venturia inaequalis*) and Apple Mildew (*Podosphaera leucotricha*) at East Malling. Journ. Pom. & Hort. Sci., 1932, **10**, 271.

(26) ———. Spraying and Dusting Experiments on the Control of Apple Scab (*Venturia inaequalis*) and Apple Mildew (*Podosphaera leucotricha*) at East Malling in 1931-2. Journ. Pom. & Hort. Sci., 1934, **12**, 57.

(27) *Le Pelley, R.* Studies on the Resistance of Apple to the Woolly Aphis. Journ. Pom. & Hort. Sci., 1927-8, **6**, 209.

(28) *Wormald, H.* and *Grubb, N. H.* The Crown Gall Disease of Nursery Stocks. I—Field Observations on Apple Stocks. Ann. App. Biol., 1924, **11**, 278.

(29) *Hatton, R. G.* and *Grubb, N. H.* Field Observations on the Incidence of Leaf Scorch upon the Apple. Journ. Pom. & Hort. Sci., 1924-5, **4**, 65.

(30) *Wallace, T.* Leaf Scorch on Fruit Trees. Journ. Pom. & Hort. Sci., 1927-8, **6**, 243 and 1928-9, **7**, 1.

(31) *Amos, J.*, *Hatton, R. G.* and *Hoblyn, T. N.* The Response of Apple Trees on Known Rootstocks to Applications of a Complete Fertilizer. Ann. App. Biol., 1930, **17**, 657.

(32) *Rogers, W. S.* and *Vyvyan, M. C.* The Root Systems of some Ten-Year-Old Apple Trees on Two Different Rootstocks, and their Relation to Tree Performance. East Malling Res. Sta. Ann. Rpt. for 1926-7 (II Supplement), 31.

(33) *Knight, R. C.* The Influence of Winter Stem Pruning on Subsequent Stem and Root Development of the Apple. Journ. Pom. & Hort. Sci., 1934, **12**, 1.

(34) *Vyvyan, M. C.* The Distribution of Growth between Roots, Stems and Leaves in a Young Apple Tree and Its Possible Bearing on the Problem of Stock Effect on Tree Vigour. East Malling Res. Sta. Ann. Rpt. for 1933 (1934), 122.

(35) *Amos, J.*, *Hatton, R. G.*, *Hoblyn, T. N.* and *Knight, R. C.* The Effect of Scion on Root. II—Stem Worked Apples. Journ. Pom. & Hort. Sci., 1930, **8**, 248.

(36) *Swarbrick, T.* and *Roberts, R. H.* The Relation of Scion Variety to Character of Root Growth in Apple Trees. Univ. of Wisconsin Expt. Sta. Res. Bull., No. 78, 1927.

(37) *Vyvyan, M. C.* The Effect of Scion on Root. III—Comparison of Stem and Root Worked Trees. Journ. Pom. & Hort. Sci., 1930, **8**, 259.

(38) *Rogers, W. S.* Root Development Investigations. East Malling Res. Sta. Ann. Rpt. for 1933 (1934), 29 ; and East Malling Res. Sta. Ann. Rpt. for 1934 (1935), 36.

(39) *Beakbane, A. B.* Analysis of Rootstock Influence, including the Reciprocal Effect of Scion on Root. East Malling Res. Sta. Ann. Rpt. for 1934 (1935), 34.

(40) *Knight, R. C.* Preliminary Observations on the Causes of Stock Influence in Apples. East Malling Res. Sta. Ann. Rpt. for 1925 (II Supplement) (1927), 51.

(41) ———. Further Observations on the Parts Played by Root and Stem in Stock Influence. East Malling Res. Sta. Ann. Rpt. for 1933 (1934), 114.

(42) *Hatton, R. G.* The Influence of Vegetatively Raised Rootstocks upon the Apple, with special Reference to the Parts Played by the Stem and Root Portions in Affecting the Scion. Journ. Pom. & Hort. Sci., 1931, **9**, 265.

(43) *Hatton, R. G.* and *Amos, J.* Analysis of Rootstock Influence, including the Reciprocal Effect of Scion on Root. East Malling Res. Sta. Ann. Rpt. for 1934 (1935), 34.

(44) *Grubb, N. H.* Fruit Research, I.B. Double Working of Apples and Pears. East Malling Res. Sta. Ann. Rpt. for 1923, 83.

(45) *East Malling Res. Sta. Annual Reports for* 1922 *and* 1923. Report on Physiology in Relation to Rootstocks. 24 and 87.

(46) *Knight, R. C.* Water Relations of Stocks and Scions. East Malling Res. Sta. Ann. Rpt. for 1924, 103.

(47) *Hafekost, G.* Über die Beziehungen zwischen Edelreis und Unterlage bei einigen Obstarten. Die Gartenbauwissenschaft, 1933, **7**, 382.

(48) *Roach, W. A.* The Chemistry of the Rootstock-Scion Effect. I—The Elements Absorbed from the Soil. East Malling Res. Sta. Ann. Rpt. for 1928-30 (II Supplement), 101.

(49) *Hatton, R. G.* Fruit Growing in the Empire. Standardization of Horticultural Material with Special Reference to Rootstocks. Memorandum and Questionnaire, 1927. Issued by the Empire Marketing Board.

(50) ———. The Development of a Research Programme around the "Build-up" of a Fruit Plant. Proceedings of IXth International Hort. Congress, London, 1931.

(51) ———. The Elimination of Sources of Error in Field Experiments. The Standardization of Fruit Tree Stocks. East Malling Res. Sta. Ann. Rpt. for 1928-30 (II Supplement) (1931), 13.

PRACTICAL BULLETINS.

(52) *Knight, R. C., Amos, J., Hatton, R. G.* and *Witt, A. W.* The Vegetative Propagation of Fruit Tree Rootstocks. East Malling Res. Sta. Ann. Rpt. for 1926-7 (II Supplement) (1928), 11.

(53) *Hatton, R. G.* A Summary of the Results Obtained in Selecting and Propagating Paradise Stocks. Gard. Chron., 1919, **65**, Nos. 1677-9.

(54) ———. Suggestions for the Right Selection of Apple Stocks. Journ. Roy. Hort. Soc., 1920, **45**, 257.

(55) ———. The Importance of Suitable Stocks in Planting Young Fruit Trees. Journ. Kent Farmers' Union, 1921, **10**, 82.

(56) *Hatton, R. G., Amos, J.* and *Grubb, N. H.* The Choice of Young Fruit Trees. East Malling Res. Sta. Ann. Rpt. for 1924 (1925), 93.

(57) *Hatton, R. G.* Apple Rootstocks, their Particular Suitabilities for Different Soils, Varieties and Purposes. East Malling Res. Sta. Ann. Rpt. for 1925 (1926) (I General), 46.

(58) *Hatton, R. G.* and *Rogers, W. S.* The Importance of the Root System. Empire Marketing Board, 1929. (Imperial Fruit Show Leaflet.)

(59) *Hatton, R. G.* Some Important Considerations in Modern Commercial Fruit Planting. Journ. Roy. Hort. Soc., 1934, **59**, 18.

(60) ———. East Malling's Experiences with Apple Rootstocks. East Malling Res. Sta. Ann. Rpt. for 1934 (1935), 223.

(*Received* 23/10/35.)

APPENDIX.

Table (*a*).

Lane's Prince Albert (Plot 11*). Accumulated Total Crops in Kilograms per tree.*

Stock	No. of Trees.	1920-24	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934
I	20*	2	11	11	21	34	100	139	196	267	**371**	439
II	20*	3	10	11	18	26	76	106	142	181	267	358
III	19	2	8	9	17	27	73	105	144	199	265	319
IV	9	2	14	14	23	**42**	**116**	**181**	**220**	**294**	370	440
V	20	1	7	7	11	20	53	83	105	148	206	259
VI	20†	1	6	7	14	27	93	132	170	228	324	420
VII	19	5	**17**	17	28	40	95	145	183	246	301	353
VIII	8	5	15	15	27	30	71	87	126	147	194	220
IX	20*	**8**	**17**	**18**	**29**	33	67	93	119	150	180	204
X	20	1	11	11	24	40	88	128	163	227	269	318
XI	9	1	12	12	23	37	93	134	174	236	305	361
XII	29	1	2	2	5	10	49	93	101	148	213	290
XIII	20	—	6	7	16	31	81	128	157	221	278	350
XIV	8	—	5	5	9	18	65	113	132	192	262	323
XV	18	—	5	5	12	24	73	111	135	201	257	321
XVI	20*	—	3	3	10	24	83	144	176	254	335	443
A	5	1	8	9	18	30	100	163	203	278	**371**	441
C	4	—	7	7	11	23	89	147	179	249	357	**455**
E	4	1	5	5	8	20	59	115	121	162	239	322
F	4	—	2	2	6	18	56	102	122	185	245	315
H	2	2	8	8	12	17	53	74	94	136	182	217
OF5	4	—	3	3	8	25	82	139	177	262	332	412

* Note: In 1934 the crops were thinned for the first time. Ten trees each on Nos. I, II, IX and XVI were left unthinned; in this summary the average crop of the ten thinned trees only is included.

† In 1931 eight trees on No. VI were blossom thinned, since that year the means of the twelve control trees only are included.

APPENDIX.

Table (*b*).

Bramley's Seedling (Plot 12). Accumulated Total Crops in Kilograms per tree.

Stock	No. of Trees	1920-24	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934
I	8	4	13	17	20	64	158	220	296	349	411	522
II	8	5	13	18	22	71	167	**246**	317	**389**	**444**	**584**
III	8	2	6	9	11	51	139	200	264	314	352	458
IV	8	5	10	15	17	64	**170**	233	**327**	361	**444**	552
V	8	2	8	10	11	39	81	162	171	251	255	373
VI	8	3	7	10	11	41	115	171	214	262	299	401
VII	8	4	10	15	17	68	161	233	273	310	346	449
VIII	3	8	15	21	23	82	109	169	174	230	249	329
IX	8	**11**	**18**	**31**	**37**	**93**	156	220	237	279	284	362
X	8	1	4	5	7	39	114	162	215	243	313	407
XI	4	2	7	10	12	70	140	224	243	335	351	493
XII	8	—	1	2	2	16	73	138	187	247	279	385
XIII	8	—	—	3	3	24	76	133	160	242	271	391
XIV	3	1	2	4	5	52	108	171	182	267	278	400
XV	8	1	2	4	5	32	98	152	206	263	318	423
XVI	8	—	2	4	5	40	93	180	192	293	309	447
E	3	1	4	6	7	32	108	128	181	210	297	407
F	4	—	1	2	3	34	91	129	162	244	294	434
OF5	4	—	—	1	1	45	97	171	181	283	320	458

APPENDIX.

TABLE (*c*).

Worcester Pearmain (*Plot* 13). *Accumulated Total Crops in Kilograms per tree.*

Stock	No. of Trees	1920-24	1925	1926	1927	1928	1929	1930	1931	1932
I	8	2	5	5	21	37	68	91	126	151
II	8	2	5	6	18	36	57	75	95	113
III	8	1	3	4	17	36	55	70	88	107
IV	8	**3**	**7**	**8**	**25**	44	80	110	138	170
V	8	—	2	2	10	22	40	50	65	82
VI	8	1	2	3	11	23	47	58	85	105
VII	8	1	3	4	13	23	44	58	86	103
IX	8	3	**7**	**8**	19	29	57	72	103	126
X	8	1	3	4	12	24	49	62	93	114
XI	7	2	6	8	23	**46**	**88**	**117**	**161**	**194**
XII	8	1	2	2	12	30	50	69	86	107
XIII	8	—	1	2	10	23	39	50	71	90
XIV	3	1	2	4	13	26	50	70	91	116
XV	8	1	2	2	13	29	49	70	100	130
XVI	8	1	2	3	13	32	52	75	92	121
E	2*	—	1	1	7	14	32	48	52	58
F	3	1	2	2	7	19	39	60	79	104
OF5	6	1	3	4	19	37	58	84	109	141

* One tree died in 1930.

APPENDIX.

Table (*d*).

Cox's Orange Pippin (*Plot* 32). *Accumulated Total Crops in Kilograms per tree.*

Stock	No. of Trees	1922-26	1927	1928	1929	1930	1931	1932	1933	1934
I	16	—	1	1	6	15	17	23	44	65
II	16	—	1	2	6	15	16	23	51	85
III	16	—	—	1	4	14	15	18	38	57
IV	8	—	—	—	2	11	18	**34**	**68**	**112**
V	16	—	—	—	2	8	10	17	43	72
VI	14	—	—	—	2	6	7	11	20	46
IX	16	**1**	**4**	**5**	**10**	**20**	**24**	27	49	72
X	15	—	—	—	1	5	6	10	34	65
XIII	16	—	—	—	1	9	10	16	33	57
XV	8	—	—	—	—	1	2	5	18	44
XVI	16	—	—	—	—	5	5	7	27	53

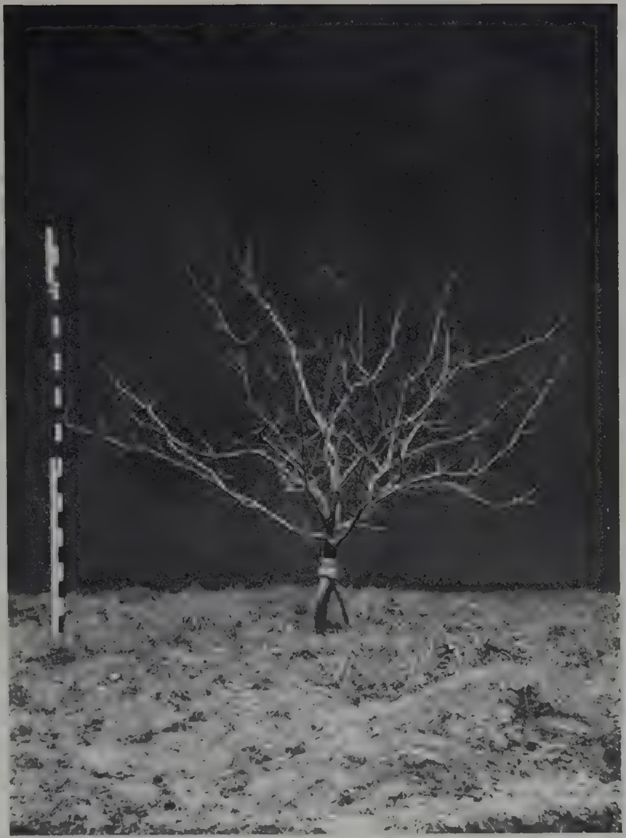

FIG. 1. Worcester Pearmain 16 years old on No. IX rootstock.

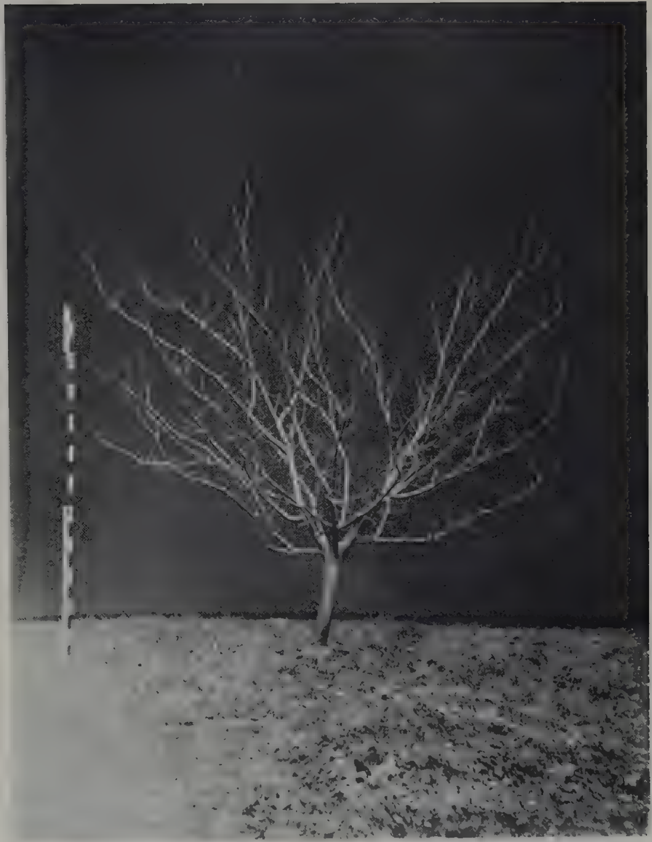

FIG. 2. Worcester Pearmain 16 years old on No. VII rootstock.

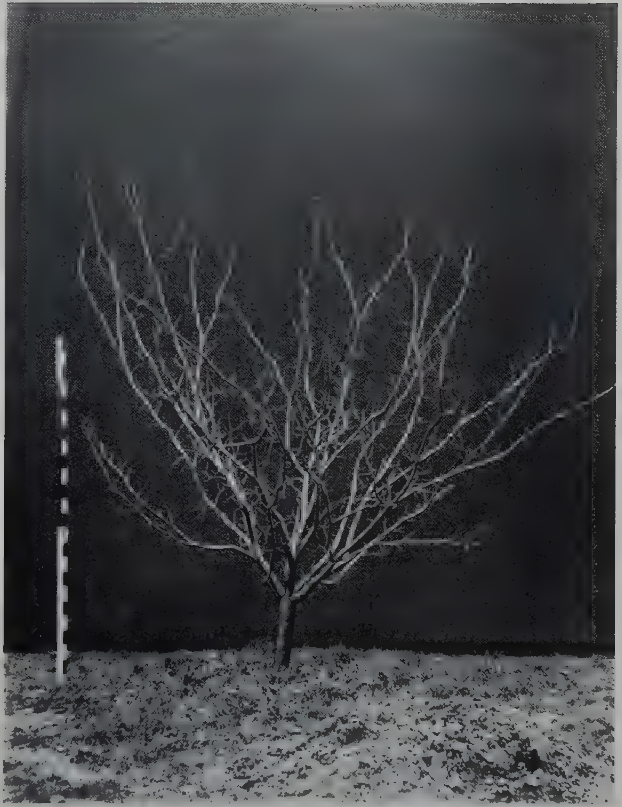

FIG. 3. Worcester Pearmain 16 years old on No. IV rootstock.

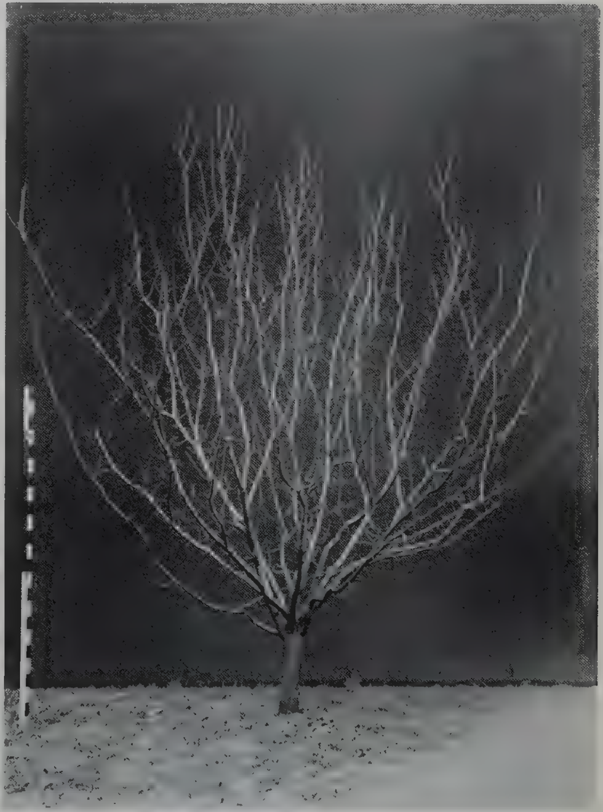

FIG. 4. Worcester Pearmain 16 years old on No. II rootstock.

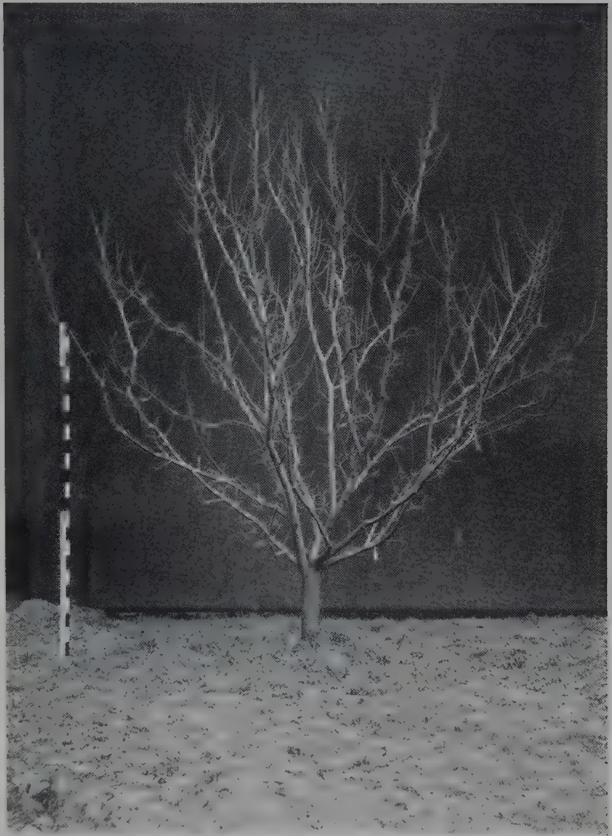

FIG. 5. Worcester Pearmain 16 years old on No. I rootstock.

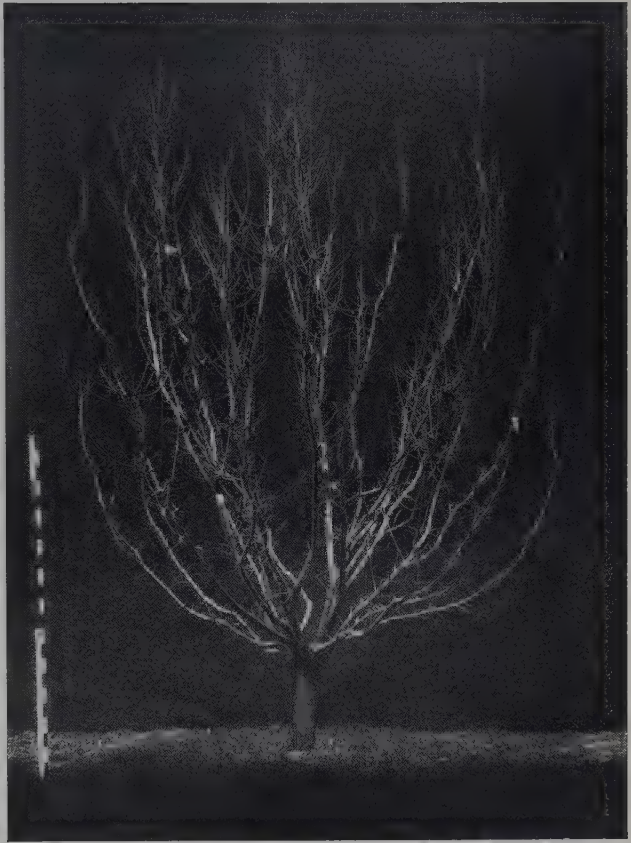

FIG. 6. Worcester Pearmain 16 years old on No. XVI rootstock.

THE FORMATION OF ETHYLENE BY PLANT TISSUES, AND ITS SIGNIFICANCE IN THE RIPENING OF FRUITS

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It is now known that ripe apples give off some vapour or gas which induces delayed and abnormal sprouting in potatoes (1). It has also been observed that ripe apples, pears, peaches, tomatoes and bananas (2, 3) produce a vapour or gas which has the effect of immediately stimulating the onset of ripening and the climacteric rise in respiratory activity in immature fruit. The object of the present enquiry has been to establish the chemical identity of this "active" substance.

The two effects of the "active" substance are paralleled closely by effects produced by ethylene in high dilution in air (Huelin (4) potato, Kidd and West (2) fruit ripening). It seemed obvious therefore at the outset that an attempt should be made to see whether other well known effects of ethylene on vegetable tissues could also be produced by the "active" unknown given off by ripe fruits.

I. Biological Analysis.

Two classes of ethylene effect were chosen (*a*) the effects upon the epinastic reactions of leaves, (*b*) the effects upon seed germination and growth.

The plants and seedlings used are listed below:

Plant tissues which are sensitive to ethylene and the volatile products from Apples.

Plants.	*Seedlings.*
Mimosa pudica (5).	Pisum sativum (9).
Ricinus communis (6).	Lathyrus odorata (9).
Helianthus (7, 8).	Radish, Swede, White Turnip, White Mustard, Mangold, Cress, Peanut, Linseed, Broad Bean, Runner Bean.
Salvia sp. (5).	
Solanum Lycopersicum (7, 8).	

Plant tissues which are insensitive to ethylene and the volatile products from apples.

Plants.	*Seedlings.*
Hedera sp. (7).	Barley, Maize, Oat, Wheat.

It was found that tissues affected by ethylene in low concentrations, of the order of 1 p.p.m., and by propylene and acetylene at much higher concentrations were affected in an identical manner by the "active substance" from apples and further that tissues which were unaffected by ethylene were also unaffected by the "active" unknown. It may perhaps be marked in parenthesis that it appears to be a new observation that cereal seedlings are insensitive to ethylene.

Tests to determine whether any known constituent of apple vapour or chemical related substances possess the properties of the "active" substance.

Ripe apples are known to form a number of substances which escape as vapour including esters, ethyl alcohol, acetaldehyde, and essential oils.

Pea seeds, sown on wet cotton wool in Petri dishes, were exposed in six-litre desiccators, to the vapour of an aqueous solution of the substance to be tested. Two hundred ml. of solution were employed, and renewed every three days. The vapour from the following solutions did not modify growth in the same manner as ethylene or the active substance from apples.

	Concentration of Aqueous solution.
Esters.	
Methyl formate	M/1,000
Methyl acetate	M/10,000
Methyl acetate	M/1,000
Methyl propionate	M/1,000
Methyl butyrate	M/1,000
Methyl isovalerate	M/1,000
Methyl salicylate	M/10,000
Ethyl acetate	M/10,000
Ethyl acetate	M/1,000
Amyl formate	M/10,000
Amyl acetate	M/10,000
Amyl butyrate	M/10,000
Alcohols.	
Methyl alcohol	M/100,000
Methyl alcohol	M/10,000
Methyl alcohol	M/1,000
Ethyl alcohol	M/100,000
Ethyl alcohol	M/10,000
Ethyl alcohol	M/1,000
Amyl alcohol	M/100,000
Amyl alcohol	M/10,000
Amyl alcohol	M/1,000
Allyl alcohol	M/1,000

Miscellaneous substances (concentration measured as v.p.).

Acetaldehyde	$0{\cdot}014 \times 10^{-3}$ atmos.
	$0{\cdot}027$
	$0{\cdot}136$
	$0{\cdot}408$
	$1{\cdot}36$
Acrolein	$6{\cdot}6 \times 10^{-3}$
Allyl alcohol	$5{\cdot}5 \times 10^{-3}$
Amylene	$5{\cdot}0 \times 10^{-3}$
Pentane	$5{\cdot}0 \times 10^{-3}$

Growth could be inhibited by acrolein and allyl alcohol at vapour pressures, of $6{\cdot}6 \times 10^{-3}$, and $5{\cdot}5 \times 10^{-3}$ atmos. respectively. By increasing the concentration of acetaldehyde, growth could be restricted until at a vapour pressure of $1{\cdot}36 \times 10^{-3}$ atmos., it was completely inhibited.

II. Chemical Tests.

Pea seedlings were used as an indicator for the presence of the active substance in a stream of air which had been passed over ripe apples, and then through one or more reagents. The results as set out below, show that only strong oxidizing agents were capable of reducing the concentration of the active substance below the toxic limit.

1. *Stability.* When an air-stream containing the active substance from apples was passed through distilled water, a sufficient amount usually escaped absorption to produce the characteristic effect on pea seedlings. When, however, peas were grown in a desiccator above this water, the abnormalities were again produced. This, at once, establishes the fact that the substance in question is stable and does not decompose in aqueous solution or in moist air.

2. *Removal by combustion.* The active substance was destroyed by passing the gas stream over copper oxide, obtained from copper-gauze previously oxidized in a stream of air, and heated in an electric furnace at 800-900° C.

Air from a cylinder was passed at the rate of 30 c.c./min. through a train of vessels as follows:

Water⟶peas A⟶single apple⟶peas B⟶electric furnace⟶peas C.

The experiment was conducted at room-temperature in diffuse daylight. After thirteen days, the seedlings A and C had germinated and were growing normally; the seedlings B showed the characteristic abnormalities.

The method of experiment rules out any possibility that the effect on seedlings B may be due to a deficiency of oxygen, or to an excess of carbon dioxide.

This apparatus was then used to measure the amount of carbon dioxide produced by the combustion of the volatile products from the fruit. At the rate of ventilation used, the concentration of volatile matter, expressed as carbon dioxide was of the order of one in 28,000 volumes of air.

3. *Reaction with Bromine.* The stream of air (30 c.c./min.) was passed successively through the following train:

Peas A⟶single apple⟶peas B⟶soda lime⟶peas C⟶saturated bromine-water⟶aqueous sodium hydroxide (20 per cent.)⟶peas D.

The sodium hydroxide was introduced to remove bromine vapour carried over by the gas-stream.

After thirteen days, the seedlings A and D had germinated, and were growing normally; B and C showed the characteristic abnormality.

It follows that the active substance is not removed below the limit of toxicity by soda-lime; but it is removed by bromine. Combination of bromine with the sodium hydroxide solution would form an alkaline solution of bromide, hypobromite and bromate. To test the possibility that the absorption or inactivation of the active substance was due to this solution, the following train was set up:

Peas A⟶apple⟶peas B⟶solution⟶soda-lime⟶peas C.

The same apple was used, and the actual alkaline solution formed in the previous experiment. The seedlings were examined after fifteen days. Both B and C were abnormal, thus the active substance is not removed by the products of reaction of bromine with sodium hydroxide.

4. *Removal by ozone.* A current of air (30 c.c./min.) was passed through a train of vessels at 25° C., in the following order:

Peas A⟶apple⟶peas B⟶10 per cent. aqueous sodium hydroxide⟶peas C⟶
⟶soda lime⟶solid calcium chloride⟶ozoniser⟶10 per cent. aqueous potassium iodide⟶10 per cent. aqueous sodium hydroxide⟶peas D.

The calcium chloride tube was included since the ozoniser operates more efficiently with dry air. The potassium iodide solution was used to remove the excess of ozone, and the sodium hydroxide which followed it served to absorb any iodine carried over in the air-stream. The first sodium hydroxide served as a check, to show whether the active substance was absorbed by this reagent itself. A separate test showed that the ozoniser yielded about 200 parts per million of ozone, under the conditions of the experiment. The connections from the ozoniser to the bottle containing the potassium iodide solution, were made by cementing a wide glass tube over the joint, with plaster of Paris; and then covering the exposed plaster with vaseline.

After ten days, the seedlings were examined with the following results: Lot A Normal growth, Lot B Abnormal growth, Lot C Abnormal growth, Lot D Normal growth.

Thus the substance is inactivated by that part of the gas-train inserted between peas C and peas D. A second experiment with the same series of vessels and solutions, but with the ozoniser out of action showed that the effect was entirely due to reaction with ozone.

It having been established that the active substance would react with ozone, attempts were made to collect the decomposition-products in distilled water. Air from a cylinder was passed (60 c.c./min.) over about 1·5 kilos. of Newtown Pippin apples, in a desiccator at 25° C., and then after being dried with calcium chloride, was led through the ozoniser and into distilled water. After twenty-four hours formaldehyde was detected by the Schryver-Rimini test. The test was negative except when the ozoniser was in action. The presence of formaldehyde suggests a terminal methylene group in the molecule of the active substance, but is not conclusive evidence, since methyl esters give rise to formaldehyde under similar conditions.

5. *Removal by fuming acids.* The following train was set up:

Peas A ⟶ apples ⟶ peas B ↗ fuming nitric acid ⟶ NaOH ⟶ peas C.
Peas A ⟶ apples ⟶ peas B ↘ fuming sulphuric acid ⟶ NaOH ⟶ peas D.

The fuming acids were contained in Pettenkofer tubes with special glass connections. After twenty days the condition of the seedlings was as follows: Lot A Normal growth, Lot B Abnormal growth, Lot C Normal growth (there was a smell of nitric acid in the bottle), Lot D Normal growth.

6. *Reagents which fail to effect complete removal.* In the above experiments the reagents which did not remove the active substance were soda lime, 20 per cent. sodium hydroxide, solid calcium chloride, iodine in potassium iodide, and sodium hypobromite. Separate tests showed that aqueous sodium bisulphite was also ineffective.

The characteristic abnormalities in seedling-growth were still produced by a gas-stream which initially contained the active substance, after it has been passed through any of the following:

Alkaline potassium permanganate (15 per cent. sodium hydroxide saturated with potassium permanganate);
Acid potassium permanganate (10 per cent. sulphuric acid saturated with potassium permanganate);
Palladium chloride (1 per cent. aqueous solution);
Ammoniacal silver oxide (10 per cent. aqueous solution of silver nitrate and excess of ammonia).

The two latter solutions were tried as possibly affording methods of estimation.

Thus a number of the milder oxidizing agents, which react with ethylene, fail to remove the active substance below the limit of toxicity to pea seedlings. It was shown, however, by separate experiment, that when ethylene (about

0·1 per cent. in air) is passed through the same reagents and under the same conditions, a sufficient amount escapes absorption to produce the characteristic effect.

III. CHEMICAL IDENTIFICATION OF THE ACTIVE SUBSTANCE.

A convenient series of reactions, giving eventually a solid crystalline product that can be readily identified, is the following:

ethylene + bromine ⟶ ethylene dibromide (M.W. 188; M.P. 9° C.; B.P. 132° C.).
(M.W. 28.)
ethylene dibromide + aniline ⟶ NN′ diphenyl-ethylene-diamine (M.W. 212; M.P. 62·5° C.).

If diphenyl-ethylene-diamine could be isolated from the final reaction-mixture of a series that started with the gaseous products from apples, this would be conclusive proof that it is ethylene which is produced by the fruit.

About 30 Kg. of English Worcester Pearmain apples were ventilated with air from cylinders, at the rate of 50 to 70 litres an hour.* The gas-stream from the fruit container was passed through two tubes of calcium chloride, and then through two "Newth" collecting tubes containing bromine, cooled in solid carbon dioxide. At this temperature (about −65° C.) the bromine is solid, but still has a vapour pressure sufficient for a reaction with low concentrations of ethylene in the gaseous phase, and probably the yield is favoured by the low temperature (10). If ethylene dibromide is formed in an amount greater than corresponds with its vapour-pressure at this temperature, condensation will occur, and the material can be collected. The experiment was continued for thirty days, and in all about 30,000 litres of air was passed through the apparatus. The gas-stream issuing from the collecting tubes had an odour reminiscent of amyl alcohol, and contained traces of bromine.

Finally, the collecting tubes were allowed to warm, and the contents were poured into distilled water. The excess of bromine was neutralized with sodium carbonate, the product was extracted with ether, and the ether-extract was dried with anhydrous sodium sulphate. After distilling off the ether, there remained a small quantity of pale yellow oily liquid with a fruity odour. When this was distilled, 0·65 g. of oil came over between 57° C. and 140° C. Between 140° C. and 160° C. only 0·07 g. of liquid was collected; above 160° C. decomposition started to occur; the residue in the flask was 0·1 g.

The first fraction did not solidify or crystallize when cooled to −20° C. It was heated with six times its weight of aniline, in a test-tube, in the water bath, for four hours. After the addition of 10 cc. of water, it was distilled in steam to remove the excess of aniline. The steam distillate had a very fruity odour, but was not examined. The residue in the flask solidified to a red, hard mass. It was extracted with chloroform, the chloroform was boiled off, and the product was extracted with hot dilute alcohol. Lustrous plates crystallized out from the dilute alcohol, and melted at 62·5° C. When mixed with an authentic specimen of diphenyl-ethylene-diamine, the mixture again melted at 62·5° C.

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* Compressed air from cylinders was used in preference to laboratory air. The compressed air gave no reaction for ethylene or any other active substance, when tested upon unripe fruits.

The picrate of this base is not recorded in the literature. Attempts to prepare it gave an orange coloured product which did not crystallize readily from dilute alcohol, and melted at about 129°C., while that from the apple-product had a melting point of 119° C. The oxalate crystallized as needles from dilute alcohol, and decomposed on melting at 185°-186° C. A specimen from the apple-product was also decomposed at 185° C. The identity of the prepared product with diphenyl-ethylene-diamine may therefore be considered as completely established. This in turn identifies the oily distillate as ethylene dibromide, and may be taken as proof of the production of ethylene by apples.

Discussion.

The data described above establish the presence of ethylene among the gaseous substances produced by ripe apples.

With regard to certain other fruits, while it has not yet been conclusively shown that they produce ethylene, the facts so far established are that ripe pears, peaches and tomatoes produce an "active" substance which affects ripe fruit in the same way as does ethylene; and that the same is true of bananas. Tests on seeds however indicate that the quantity of the active substance produced by ripe bananas is much smaller than in the case of apples (11).

It is interesting to consider a possible function of the active substance. Prior to the climacteric rise in respiratory activity which marks the onset of ripening, apples and bananas have been shown to be sensitive to an active substance which these fruits will produce once ripening has begun. Subsequent to the beginning of the climacteric rise in respiratory activity they are no longer sensitive. The change induced by the "active" substance thus appears to be a final and irreversible one.

From the above it follows that if once the critical change has occurred in any part of the tissue, it must through the action of the "active" substance, be rapidly propagated throughout the whole fruit and further since the active substance is volatile it must spread to neighbouring fruits.

SUMMARY.

Ripe apples generate a gaseous substance, which produces epinastic effects on the petioles and leaves of growing plants similar to the effects produced by low concentrations of ethylene.

The growth of many seedlings, but not of cereal seedlings, is abnormal in the presence of the active substance from apples, and the abnormalities are similar to those produced by low concentrations of ethylene. Cereals, however, do not show any abnormal type of growth in air containing 10 p.p.m. of ethylene.

This abnormal growth of seedlings is not produced in low concentrations of simple esters identical with or similar to those evolved by ripe apples.

The active substance from apples is completely removed by combustion and by reaction with bromine, ozone, fuming nitric acid, and fuming sulphuric acid.

The reaction with bromine yields an oil, which can be identified as ethylene dibromide by the formation of diphenyl-ethylene-diamine with aniline. This supplies chemical proof of the identity of the active substance with ethylene.

REFERENCES.

(1) *Elmer, O. H.* Growth inhibition of potato sprouts by the volatile products of apples. Science 1932, **75**, 193.

(2) *Kidd, F.* and *West, C.* Effects of ethylene and of apple vapours on the ripening of fruits. Food Invest. Bd. Ann. Rpt., 1932, 55.

(3) ——— ———. The Influence of the composition of the atmosphere upon the incidence of the climacteric in apples. Food Invest. Bd. Ann. Rep., 1933, 51.

(4) *Huelin, F. E.* Effects of ethylene and of apple vapour on the sprouting of potatoes. Food Invest. Bd. Ann. Rep., 1932, 51.

(5) *Doubt, S. L.* The response of plants to illuminating gas. Bot. Gaz., 1917, **63**, 209.

(6) *Harvey, E. M.* Castor beam plant in laboratory air. Bot. Gaz., 1913, **56**, 439.

(7) *Crocker, W.*, *Zimmerman, P. W.* and *Hitchcock, A. E.* The movement of gases into and through plants. Contr. Boyce Thomp. Inst., 1931, **3**, 313.

(8) ——— ——— ———. Ethylene-induced epinasty of leaves and the relation of gravity to it. Contr. Boyce Thomp. Inst., 1932, **4**, 177.

(9) *Knight, L. I.* and *Crocker, W.* Toxicity of smoke. Bot. Gaz., 1913, **55**, 337.

(10) *Balis, H.* and *Zieler, H.* Action of chlorine on ethylene. Z. augew, Chem., 1930, **43**, 233.

(11) *Gane, R.* Tainting and the volatile products of fruits. Food Invest. Bd. Ann. Rpt., 1933, 122.

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BOOK REVIEWS

THE LIVING GARDEN. By E. J. Salisbury. (Bell, London, 1935, illus., 10s. 6d.)

This book is written, it is remarked in the preface, with the object of engendering a greater interest in the lives of the garden plants around us, of stimulating a more vivid appreciation of those plants as living entities and of bringing closer the co-operation between the professional botanist and the horticulturist, each of whom perhaps may have opportunities of study and observation denied to the other. And so Professor Salisbury sets out to tell us why things happen in the garden. Why plants respond as they do and often in a manner differing with each variety, to heat and cold, light and shade, long days and short days, wind and soil moisture and to the gardener's attempts, sometimes misguided, at assisting them. Now there are innumerable gardening books to tell you *how*, and precious dull some of them are, but those that tell you *why* are few indeed, and fewer still those that can do so in a manner to be understanded of the people. We venture to think that although the reader's botanical investigations may hitherto have proceeded no further than the uninterested dissection of a cabbage at the dinner table he would nevertheless have no difficulty in understanding and appreciating every word in this book. Yet if there are no cryptic technicalities neither are there long or laboured explanatory substitutes. In that apparently effortless manner which marks the artist, without the slightest hint of talking down, yet with a lucidity of expression that should penetrate the woolliest mind, the hidden mysteries of nature are laid before us, and, we had almost said, " debunked ". The author, however, quoting Shelley, prefers much more appropriately to describe his work as an attempt to " lift the veil from the hidden beauties of the world ". He has well succeeded. That quotation symbolizes the spirit in which the author has approached his task, the spirit which has enabled him to infuse the vital spark into the dry bones of his scientific facts, and to clothe those bones with beauty. The enquiring layman, possibly approaching the book with an apprehension born of past experiences, will need to peruse but few pages before discovering with gratitude that here indeed is that *rara avis*—a scientist who can *write*. It might be added that the lady who has provided the illustrations can draw.

G.St.C.F.

INSECT PESTS OF GLASSHOUSE CROPS. By Herbert W. Miles and Mary Miles. (H. C. Long, 1935, 8s. 6d.)

During the last twenty years a number of works have been published on systematic entomology, but the economic aspect has been somewhat neglected.

It is a pleasure, therefore, to note that a book on the insect pests of glasshouse crops has been compiled at last.

In a Foreword to the book Mr. J. C. F. Fryer rightly points out that since the plants are growing under glass they are in an artificial climate, thus the insects which attack them may differ from those out of doors and being in a heated house may live for a much longer period. Finally it is pointed out that new insects may be introduced with hot house plants and these should be reported to a competent authority.

Dr. and Mrs. Miles have been associated with investigations on the control of glasshouse pests for many years, and it is very fitting that they should be the authors of a book which will prove of immense value to all those who specialize in growing glasshouse plants, to the Nurseryman, and also to Advisory and Research Entomologists. In addition the book is written in such a manner that while its scientific value is not lost, at the same time it is very suitable for the layman and doubtless it will find its way in to the homes of many amateur gardeners.

It is divided into nine chapters each dealing with a special aspect of the problem. For example Chapter IV deals with the Aphides, Capsis and Leaf Hoppers ; while Chapter IX describes Methods of Pest Control in glasshouses.

The eighty figures in twenty-one plates are excellent and very instructive since many of them illustrate the type of damage caused by the pest concerned, thus the common fault of figuring the pest without showing the damage caused by it has been overcome. An error appears in Plate XVII, Figs. 72 and 74 should be transposed. One wonders why the authors have decided to include mushroom mites as pests of glasshouse crops, but since they have it is a pity that more detailed information is not given. The subject is dismissed in little more than half a page, which is very little considering the recent research carried out in the United States and in this country.

The arrangement of the book is very good and reference to any crop or pest can be obtained at a glance. This has been rendered possible by the very complete index, which is supplemented by an alphabetical list of crops with associated pests and their type of injury. A selected bibliography completes the book.

A.M.M.

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